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Draft Ambient Water Quality Criteria for Dissolved Oxygen (Saltwater): Cape Cod to Cape Hatteras

Ambient Water Quality Criteria for Dissolved Oxygen (Saltwater): Cape Cod to Cape Hatteras

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Office of Research and Development
National Health and Environmental Effects Research Laboratory
Atlantic Ecology Division
Narragansett, Rhode Island

Notices

This document provides guidance to States and Tribes authorized to establish water quality standards under the Clean Water Act (CWA) concerning dissolved oxygen values that protect aquatic life from acute and chronic effects. Under the CWA, States and Tribes are to establish water quality criteria to protect designated uses. State and tribal decision-makers retain the discretion to adopt approaches on a case-by-case basis that differ from this guidance when appropriate. While this document constitutes EPA's scientific recommendations regarding ambient concentrations of dissolved oxygen that protect saltwater aquatic life in the Virginian Province, this document does not substitute for the CWA or EPA's regulations; nor is it a regulation itself. Thus, it cannot impose legally binding requirements on EPA, States, Tribes, or the regulated community, and might not apply to a particular situation based upon the circumstances. EPA may change this guidance in the future.

This document has been reviewed by the Atlantic Ecology Division, Narragansett, RI (Office of Research and Development) and the Office of Science and Technology (Office of Water), U.S. Environmental Protection Agency, and approved for publication.

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Executive Summary

This document recommends a way to derive the lower limits of dissolved oxygen (DO) necessary to protect coastal and estuarine animals in the Virginian Province (Cape Cod, MA to Cape Hatteras, NC). The information on hypoxic effects used here was obtained from studies conducted by the USEPA's Atlantic Ecology Division specifically for this purpose, and from all other available reports applicable to hypoxic issues of the Virginian Province. Hypoxia is defined here as concentrations of DO that are below saturation. Literature on the effects of anoxia, while applicable to certain ecological risk analyses, was not included in this document. This approach combines features of traditional Water Quality Criteria with a new biological framework that integrates time (replacing the concept of an averaging period) and establishes separate criteria for different life stages (larvae versus juveniles and adults). Where practical, data were selected and analyzed in manners consistent with the *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (Stephan, et al., 1985). This document considers how to protect three aspects of biological health: survival of juveniles and adults, growth, and larval recruitment (estimated with a generic model).

The criteria described here apply to both continuous (persistent) and cyclic (diel, tidal, or episodic) hypoxia. If the DO exceeds the chronic protective value for growth (4.8 mg/L), the site meets objectives for protection. If the DO is below the limit for juvenile and adult survival (2.3 mg/L), the site does not meet objectives for protection. When the DO is between these values, the site requires evaluation of duration and intensity of hypoxia to determine suitability of habitat for the larval recruitment objective.

The limits identified are based entirely on laboratory findings but are supported in part by field observations. For example, juvenile and adult animals showed field acute effects at < 2.0 mg/L, below the limit of 2.3 mg/L for juveniles and adults. Also, behavioral effects were generally seen in the range of laboratory sublethal effects. Unfortunately, however, no field observations are available for survival and growth of larvae that are sensitive to hypoxia. This type of information is critical because two of the three criteria are derived from laboratory responses of larvae.

Dissolved oxygen criteria should be implemented differently from toxicant criteria, but not for reasons of biological effects or exposure. The primary reason is that DO is not regulated directly, whereas toxic compounds are. Hypoxia is a symptom of a problem not the problem itself. Dissolved oxygen is regulated primary by controlling nutrients, largely nitrogen. Dissolved oxygen also differs from most toxic compounds in that hypoxia in a given area can be largely natural. Criteria for DO can be used in a risk assessment framework. The limits presented by the approach outlined here can be easily used to compare the abilities of different areas to support saltwater aquatic life. Environmental managers can determine which sites need the most attention, and how hypoxic problems vary in time and space from one year to the next. Finally, environmental planners can make better cost-benefit decisions by using this approach to evaluate how various management scenarios will improve conditions.

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Introduction

Section 304 (a)(2) of the Clean Water Act calls for information on the conditions necessary "to restore and maintain biological integrity of all . . . waters, for the protection and propagation of shellfish, fish and wildlife, to allow recreational activities in and on the water, and to measure and classify water quality." The Environmental Protection Agency has not previously issued saltwater criteria for dissolved oxygen (DO) because the available information on effects was insufficient. This document is the result of a research effort to produce the required information to support the development of saltwater DO criteria. The criteria presented herein represent the best estimates, based on the available data, of DO concentrations necessary to protect aquatic life and its uses.

The geographic scope of this document is limited to the Virginian Province of the Atlantic coast of the United States (i.e., southern Cape Cod, MA, to Cape Hatteras, NC). The document provides the information necessary for environmental planners and regulators in the Virginian Province to decide whether the DO at a given site can protect coastal or estuarine aquatic life. The approach can be used to evaluate existing localized DO goals (e.g., Jordan, et al., 1992) or to establish new ones. This document does not address direct behavioral responses (i.e., avoiding low DO) or the ecological consequences of behavioral responses such as changes in predation rates or in community structures. The document also does not address the issue of spatial extent of a DO problem. A given site may have DO conditions expected to cause a significant effect on aquatic life, however, the environmental manager will have to judge whether the spatial extent of the low DO area is sufficient to warrant concern. The approach presented here for deriving criteria is expected to work for other regions. However, additional regionally specific data may be required in order to amend the database for use in other regions. Animals may have adapted to lower oxygen in locations where high temperatures have historically reduced concentrations, or in systems with natural high demands for oxygen. In addition, effects of hypoxia[1] may vary latitudinally, or site-specifically, particularly as reproductive seasons determine risks of exposure for sensitive early life stages.

As with the freshwater DO document (U.S. EPA, 1986), all data and criteria are expressed in terms of the actual amount of DO available to aquatic organisms in milligrams per liter (mg/L). However, unlike the freshwater document, which provides limits for DO in both warm and cold water, criteria are presented for only warm saltwater because hypoxia in Virginian Province coastal waters is primarily restricted to the warm water of summer. Also, the freshwater criteria are based almost entirely on fish data even though insects were often more sensitive than fish. The saltwater limits, on the other hand, use data from fish and invertebrates.

[1] Hypoxia is defined in this document as the reduction of DO concentrations below air saturation.

The saltwater criteria described herein are intended to maintain and support aquatic life and their designated uses. Criteria derived using the *Guidelines*[2] are intended to protect aquatic communities, but they rely primarily on data generated at the organism level, and emphasize data for the most sensitive life stage. But a population of a given species can potentially withstand some mortality to certain life stages without a significant long-term effect on the population. Hence, an assessment of criteria should include population-level considerations. One nuance of population-level assessment is the fact that a population's sensitivity to hypoxia may depend on which stages have been exposed. For example, many populations of marine organisms may be more impacted by mortality occurring during the juvenile and adult stages than during the larval stage(s). In this regard, a particular individual larva is not as important to the population as a particular individual juvenile or adult. With this in mind, the saltwater criteria for DO segregate effects on juveniles and adults from those on larvae. The survival data on the sensitivity of the former are handled in a traditional *Guidelines* manner. The cumulative effects of low DO on larval recruitment to the juvenile life stage, on the other hand, address survival effects on larvae. The recommended DO approach uses a mathematical model to evaluate the effect on larvae by tracking intensity and duration effects across the larval recruitment season. Protection for larvae of all species is provided by using data for a sensitive aquatic organism (the Say mud crab *Dyspanopeus sayi* in this case). This model is used to generate a DO criterion for larval survival as a function of time.

For the reasons listed above, the approach recommended below to derive DO criteria for saltwater animals deviates from EPA's traditional approach for toxic chemicals outlined in the *Guidelines*. Where practical, however, data selection and analytical procedures are consistent with the *Guidelines*. Therefore, some of the terminology and the calculation procedures are the same. Thus, knowing the *Guidelines* are useful (but not essential) for better understanding how the limits were derived. Terminology from the *Guidelines* used here includes Species Mean Acute Value (SMAV), Genus Mean Acute Value (GMAV), Final Acute Value (FAV), Genus Mean Chronic Value (GMCV) and Final Chronic Value (FCV). Procedures from the *Guidelines* include those for calculating FAVs, Criterion Maximum Concentration[3] (CMC) and Criterion Continuous Concentration (CCC).

Overview of the Problem

The EPA's Environmental Monitoring and Assessment Program (EMAP) for the estuaries in the Virginian Province has shown that 25% of its area is exposed to some degree to DO concentrations less than 5 mg/L (Strobel et al., 1995). EMAP has also generated field observations that correlate biological degradation in many benthic areas with low DO in the lower water column (Paul et al., 1997). The two reports serve to emphasize that low DO is a major concern within the Virginian Province. Even though hypoxia is a major concern, a strong technical basis for developing benchmarks for effects of low DO has been lacking.

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[2] *Guidelines for deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (Stephen, et al., 1985—hereafter referred to as the *Guidelines*).

[3] Although in the case of dissolved oxygen, CMC is more appropriately defined as the Criterion *Minimum* Concentration.

Hypoxia in the Virginian Province is essentially a warm-water phenomenon. In the southern portions of the Province, such as the Chesapeake Bay and its tributaries, DO may be reduced any time between May and October; in the more northern coastal and estuarine waters, any time from late June into September. Hypoxic events may be seasonal or diel. Seasonal hypoxia often develops as stratified water prevents the oxygenated surface water from mixing downward. Low DO then appears in the lower waters when respiration in the water and sediment depletes oxygen faster than it can be replenished. As summer progresses, the areas of hypoxia expand and intensify, then disappear as the water cools in the fall. The cooler temperatures eliminate the stratification and allow the surface and bottom waters to mix. Diel cycles of hypoxia often appear in unstratified shallow habitats where nighttime respiration can temporarily deplete DO.

Although the primary fauna at risk from exposure to hypoxia in the Virginian Province are summer inhabitants of subpycnocline[4] (i.e., bottom) waters, hypoxia can occur in other habitats as well. For example, upwelling may permit subpycnocline, oxygen-poor water to intrude into shallow areas. Hypoxia also may appear in the upper water of eutrophic water bodies on calm, cloudy days, when more oxygen is consumed than is produced by photosynthesis and when atmospheric reaeration is limited. In spite of this tendency, however, minima in DO are generally less severe above the pycnocline than below it. Hypoxia above the pycnocline also tends to be more transient because it largely depends on weather patterns.

Hypoxia may persist more or less continuously over a season (with or without a cyclic component) or be episodic (i.e., of irregular occurrence and indefinite duration). Continuous hypoxia without a cyclic component is exemplified in the subpycnocline waters of western Long Island Sound and off the New Jersey coast (Armstrong, 1979). Hypoxia in Long Island Sound may be interrupted temporarily by major storms, but returns one or two weeks later, when the waters again become stratified (Welsh et al., 1994).

Hypoxia may oscillate with tidal, diel or lunar frequencies. Tidal hypoxia is common in subpycnocline waters of the mesohaline Chesapeake Bay main stem and the mouth of the adjacent tributaries during summer (Sanford et al., 1990; Diaz et al., 1992). In this case, DO concentrations oscillate as the tides alternately advect poorly oxygenated subpycnocline water from the mid-bay trough or tributaries and better oxygenated water from the lower bay. Diel cycles of hypoxia are found in small eutrophic embayments and harbors all along the coast of the Virginian Province, where oxygen is depleted overnight by respiration and replenished by photosynthesis after dawn. The Childs River is an example of diel hypoxia (D'Avanzo and Kremer, 1994). Lunar cycles of oxygen may occur in various systems but have been documented most clearly at the mouths of some Chesapeake Bay tributaries, where destratification from spring tides saturates the water with oxygen and stratification afterward depletes the oxygen (Haas, 1977; Kuo et al., 1991; Diaz et al., 1992).

[4] The pycnocline is the region of density discontinuity in a stratified water column between surface and bottom waters. The density difference between the two is primarily due to differences in temperature and salinity.

Episodic hypoxia has been noted in shoal waters of mid-Chesapeake Bay (Breitburg, 1990) and in adjacent tributaries (Sanford et al., 1990). Persistent winds tilt the pycnocline laterally and displace low DO water onto the shoals or tributaries indefinitely. As noted above, DO may also be reduced episodically in eutrophic surface waters, particularly during calm and cloudy weather, when photosynthesis is slow and daytime reoxygenation is reduced.

Biological Effects of Low Dissolved Oxygen

Oxygen is essential in aerobic organisms for the electron transport system of mitochondria. Oxygen insufficiency at the mitochondria results in reduction in cellular energy and a subsequent loss of ion balance in cellular and circulatory fluids. If oxygen insufficiency persists, death will ultimately occur, although some aerobic animals also possess anaerobic metabolic pathways, which can delay lethality for short time periods (minutes to days). Anaerobiosis is well developed in some benthic animals, such as bivalve molluscs and polychaetes, but not in other groups, like fish and crustaceans (Hammen, 1976). There is no evidence that any free-living animal inhabiting coastal or estuarine waters can live without oxygen indefinitely.

Many aquatic animals have adapted to short periods of hypoxia and anaerobiosis by taking up more oxygen and transporting it more effectively to cells and mitochondria, i.e., by ventilating its respiratory surfaces more intensely and increasing its heart rate. If these responses are insufficient to maintain the blood's pH, the oxygen carrying capacity of the respiratory pigment will decrease. An early behavioral response might be moving faster toward better-oxygenated water. However, if the hypoxia persists, the animal may reduce its swimming and feeding, which will reduce its need for energy and hence oxygen. Such reduce motor activity may make the animal more tolerant over the short term, but will not solve its long-term problem. For example, even the modest reductions in locomotion required by mild hypoxia may make the animal more vulnerable to predators, and the reduced feeding may decrease its growth.

Compensatory adaptations are well developed in marine animals that commonly experience hypoxia, e.g., intertidal and tide pool animals (McMahon, 1988), and burrowing animals, which partly explains their reported high tolerance to low DO. In contrast, compensatory adaptations are poorly developed in animals that inhabit well-oxygenated environments such as the upper water column. The animals most sensitive to hypoxia are among this latter group. Details on compensatory adaptations to hypoxia are provided in reviews for marine animals (Vernberg, 1972), aquatic invertebrates (Herreid, 1980) and fish (Holeton, 1980; Hughes, 1981; Kramer, 1987; Rombough, 1988a, and Heath, 1995).

Overview of the Approach

The approach to determine the limits of DO that will protect saltwater animals within the Virginian Province considers both continuous (i.e., persistent) and cyclic (e.g., diel) exposures to low DO. The continuous situation is covered first, and deals with exposures longer than 24 hr. It is followed by sections on criteria for exposures of less than

24 hr but that may be repeated for days. Both scenarios cover three areas of protection (summarized here, and explained in more detail in the sections that follow):

1. *Juvenile and adult survival*—A lower limit is calculated for continuous exposures by using Final Acute Value (FAV) calculation procedures outlined in the *Guidelines* (Stephan et al., 1985), but with data for only juvenile or adult stages. Limits for cyclic exposures are derived from an appropriate time-to-death curve for exposures less than 24 hr.
2. *Growth effects*—A threshold above which long-term, continuous exposures should not cause unacceptable effects is derived from growth data (mostly from bioassays using larvae). This Final Chronic Value (FCV) is calculated in the same manner as the FAV for juvenile and adult survival. This threshold limit as currently presented has no time component (it can be applied to exposures of any duration). Cyclic exposures are evaluated by comparing reductions in laboratory growth from cyclic and continuous exposures.
3. *Larval recruitment effects*—A larval recruitment model was developed to project cumulative loss caused by low DO. The effects depend on the intensity and the duration of adverse exposures. The maximum acceptable reduction in seasonal recruitment was set at 5%, which is equivalent to the protective limit for juvenile and adult survival. The number of acceptable days of seasonal exposure to low DO decreases as the severity of the hypoxic condition increases. The severity of cyclic exposure is evaluated with a time-to-death model (as in the protective limit for juveniles and adults).

Persistent Exposure to Low Dissolved Oxygen

Juvenile and Adult Survival

Data were used from tests with exposure ranging from 24 to 96 hr. This maximized the number of genera for the FAV calculation. Data for juveniles show that LC50 values calculated for 24 and 96 hr observations are very similar (Figure 1), therefore, all values are applied as 24 hr data. The restriction of the data set to tests of 96 hr duration or less was somewhat arbitrary; however, 96 hr is the duration used for most acute tests for traditional water quality criteria (Stephan et al., 1985). In addition, there are insufficient test data to compare 24 hr exposures versus those longer than 96 hr. Juvenile and adult mortality data from exposures longer than 96 hr are compared to the final criterion in the section on Other Laboratory Bioassay Data.

Data on the acute sensitivity of juvenile and adult saltwater animals to low DO is available for 12 invertebrate and 11 fish species (almost all of the data are for juveniles). The values are summarized in Table 1 and Appendix B. Overall Genus Mean Acute Values (GMAVs) range from <0.34 mg/L for the green crab *Carcinus maenas* to 1.63 mg/L for the pipe fish *Syngnathus fuscus*; a factor greater than 4.8. Juvenile fish are somewhat more sensitive than juvenile crustaceans (Table 1; Figure 2). In fact, the four most sensitive genera are all fish, and the range of values for these is 1.32 to 1.63 mg/L; a ratio of only 1.2.

As stated previously, the criterion for juveniles and adults exposed to continuous low DO was calculated using the *Guidelines* procedures for derivation of an FAV

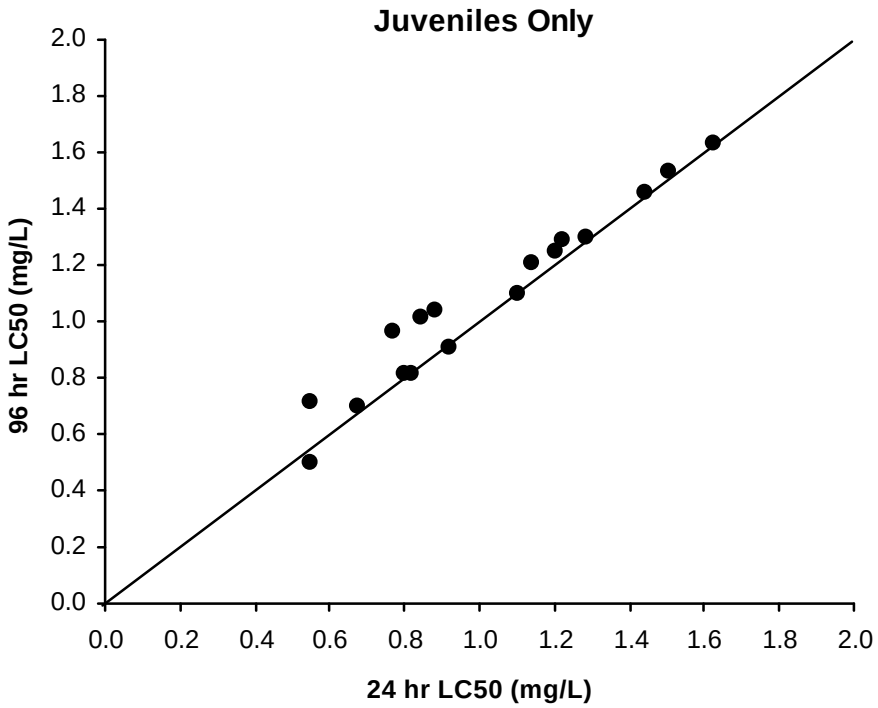


Figure 1. Relationship between 24 and 96 hr LC50 values for juvenile saltwater animals exposed to continuous low dissolved oxygen. Each point represents a paired set of values calculated from the same test run. The line drawn represents a one-to-one relationship. Data for the plot are summarized by species in Appendix A. Appendix A also contains data for test runs with larvae.

(Stephan, et al., 1985). However, the procedures outlined in the *Guidelines* were created for toxicants. Since DO behaves in the opposite manner to toxicants (i.e., the greatest response is associated with the lowest concentrations), DO concentration data were transformed by using their inverse in the calculation. The FAV calculation is essentially a linear regression using the LC50 values for the four most sensitive genera and their respective percentile ranks. The final FAV is the value representing the 5th percentile genus[5], which for DO is 1.64 mg/L. This value is adjusted to a criterion of 2.27 mg DO/L by multiplying by 1.38, the average LC5 to LC50 ratio[6] for juveniles (Table 1). This value is analogous to the CMC (Criterion Maximum Concentration) in traditional Water Quality Criteria for toxicants.

[5] Alternatively we could have modified the FAV calculation procedure to use untransformed data and established the protective limit for the 95th percentile. However, the calculated results would be the same. Since many researchers already have computer programs that calculate FAVs, we opted to remain consistent with the *Guidelines* by using the inverse data.

[6] The use of a ratio to adjust the FAV to a CMC is designed to estimate a negligible lethal effect concentration corresponding to the 5th percentile species. It may in fact represent an adverse effect concentration for species more sensitive than the 5th percentile. The *Guidelines* use a factor of 2, however, there were sufficient data available for low DO to use a factor specific to this stressor.

Table 1. Acute sensitivity of juvenile and adult saltwater animals to low dissolved oxygen. Exposure durations ranged from 24 to 96 hr. Data from individual tests are presented in Appendix B.

Species	Common name	Life Stage	SMAV LC50[a]	SMAV LC5	SMAV LC5/LC50	GMAV LC50	GMAV LC5	GMAV LC5/LC50	GMAV Rank[b]
Carcinus maenus	green crab	Juvenile/Adult	< 0.34			< 0.34			22
Spisula solidissima	Atlantic surfclam	Juvenile	0.43	0.70	1.63	0.43	0.70	1.63	21
Rithropanopeus harrisii	Harris mud crab	Juvenile	0.51			0.51			20
Prionotus carolinus	northern sea robin	Juvenile	0.55	0.80	1.45	0.55	0.80	1.45	19
Eurypanopeus depressus	flat mud crab	Juvenile	0.57			0.57			18
Leiostomus xanthurus	spot	Juvenile	0.70	0.81	1.16	0.70	0.81	1.16	17
Tautoga onitis	tautog	Juvenile	0.82	1.15	1.40	0.82	1.15	1.40	16
Palaemonetes vulgaris	marsh grass shrimp	Juvenile	1.02	1.4	1.37	0.86	1.24	1.45	15
Palaemonetes pugio	daggerblade grass shrimp	Juvenile	0.72	1.1	1.53				
Ampelisca abdita	amphipod	Juvenile	< 0.9			< 0.9			14
Scopthalmus aquosus	windowpane flounder	Juvenile	0.81	1.20	1.48	0.90	1.20	1.48	13
Apeltes quadracus	fourspine stickleback	Juvenile/Adult	0.91	1.20	1.32	0.91	1.20	1.32	12
Homarus americanus	American lobster	Juvenile	0.91	1.6	1.76	0.91	1.6	1.76	11
Crangon septemspinosa	sand shrimp	Juvenile/Adult	0.97	1.6	1.65	0.97	1.6	1.65	10
Callinectes sapidus	blue crab	Adult	< 1.0			< 1.0			9
Brevoortia tyrannus	Atlantic menhaden	Juvenile	1.12	1.72	1.53	1.12	1.72	1.53	8
Crassostrea virginica	eastern oyster	Juvenile	< 1.15			< 1.15			7
Stenotomus chrysops	scup	Juvenile	1.25			1.25			6
Americamysis bahia	mysid	Juvenile	1.27	1.50	1.16	1.27	1.50	1.16	5
Paralichthys dentatus	summer flounder	Juvenile	1.32	1.57	1.19	1.32	1.57	1.19	4
Pleuronectes americanus	winter flounder	Juvenile	1.38	1.65	1.20	1.38	1.65	1.20	3
Morone saxatilis	striped bass	Juvenile	1.58	1.95	1.23	1.58	1.95	1.23	2
Syngnathus fuscus	pipe fish	Juvenile	1.63	1.9	1.17	1.63	1.9	1.17	1

Final Acute Value=	1.64	mg/L
Mean LC5/LC50 Ratio=	1.38	
CMC = 1.64 mg/L x 1.38 =	2.27	mg/L

[a]SMAVs (Species Mean Acute Values) and GMAVs (Genus Mean Acute Values) are all geometric means (Stephan et al., 1985).

[b]Ranked by LC50 GMAV

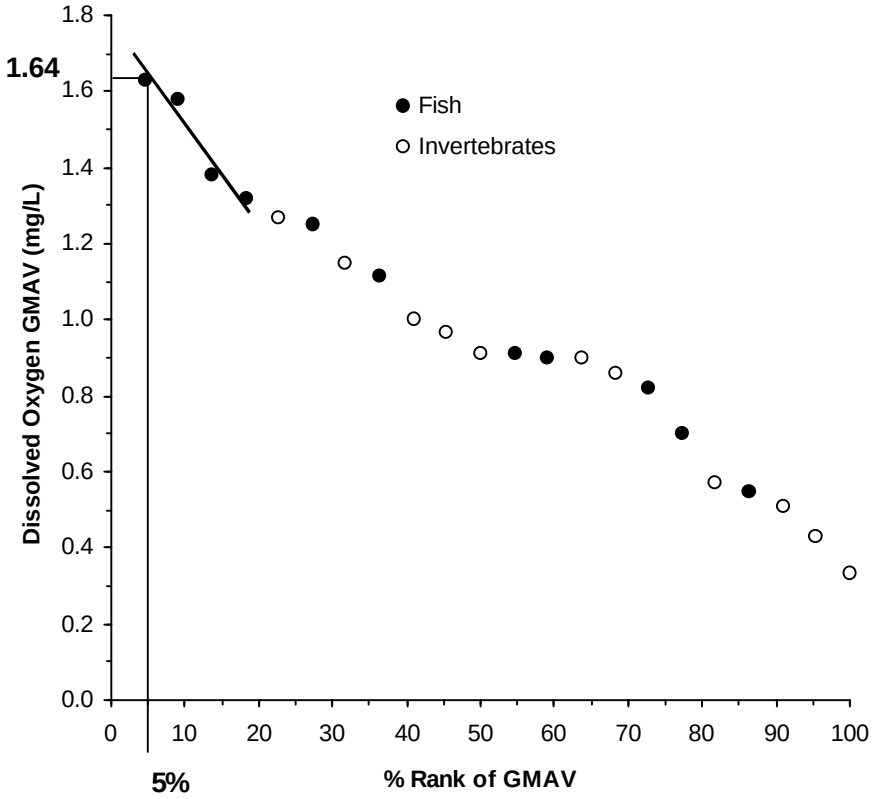


Figure 2. Plot of low dissolved oxygen effect (Genus Mean Acute Values for LC50s) against percentile rank of each value in the data set. Values for each genera are listed in Table 1. Results from individual tests for each species are listed in Appendix B. The value highlighted on the y-axis is the calculated Final Acute Value (FAV). This value is the LC50 that is higher than the values for 95% of the tested genera. The line drawn through the four most sensitive genera is the line of best fit for those four values. The LC50 values for the four most sensitive genera are the only values used in the FAV calculation other than the total number ("n") of values.

Growth Effects

A threshold above which long-term, continuous exposures to low DO should not cause unacceptable effects was calculated with growth data (mostly from bioassays using larvae). Sub-lethal effects were evaluated with only growth data for two reasons. First, growth is generally more sensitive to low DO than survival. There were only two exceptions where survival was more sensitive to low DO than growth. One test was with *D. sayi*, however, growth was the more sensitive endpoint in eight other tests with this species (Appendix C). The results from this one test were not included in Table 2. The other exception was a 28-day early life stage test using the Atlantic silverside *Menida menidia* (Appendix C). There was no effect at 4.8 mg/L DO, but there was 40% mortality and a 24% reduction in growth at a DO concentration of 3.9 mg/L. This 24% reduction in

Table 2. Effects of low dissolved oxygen on growth of saltwater animals. Data from individual tests are presented in Appendix C.

Species	Common name	Life Stage	Duration (days)	NOEC[a]	HOEC[a]	Chronic Value	Geo-Mean	Rank[b]
Cyprinodon variegatus	sheepshead minnow	larval	14	2.5	1.5	1.94	> 1.97	11
Cyprinodon variegatus	sheepshead minnow	larval	7	7.5	2.0	> 2.00		
Americamysis bahia	mysid	<48 hr old juvenile	10	2.4	1.6	1.96	2.67	10
Americamysis bahia	mysid	<48 hr old juvenile	28	4.17	3.17	3.64		
Morone saxatilis	striped bass	juvenile	21	2.8	<	2.8	< 2.8	9
Cancer irroratus	Atlantic rock crab	larval stage 5 to megalopa	7	3.42	2.41	2.87	2.87	8
Palaemonetes vulgaris	marsh grass shrimp	newly hatched	8	6.71	3.42	4.79	3.15	7
Palaemonetes vulgaris	marsh grass shrimp	<16 hr old	7	5.40	3.77	4.51		
Palaemonetes vulgaris	marsh grass shrimp	<16 hr old	8	6.94	3.20	4.71		
Palaemonetes vulgaris	marsh grass shrimp	larval stage 1 to 3	7	2.30	1.56	1.89		
Palaemonetes vulgaris	marsh grass shrimp	post larval	14	3.57	2.59	3.04		
Palaemonetes vulgaris	marsh grass shrimp	post larval	14	3.42	2.17	2.72		
Palaemonetes vulgaris	marsh grass shrimp	post larval	14	2.5	1.51	1.94		
Mercenaria mercenaria	northern quahog	embryo	14	4.2	2.4	3.17	3.17	6
Menidia menidia	Atlantic silverside	embryo to larva	28	3.9	2.8	3.30	3.30	5
Paralichthys dentatus	summer flounder	newly metamorphosed juvenile	14	4.53	3.53	4.00	3.97	4
Paralichthys dentatus	summer flounder	newly metamorphosed juvenile	14	4.39	3.39	3.86		
Paralichthys dentatus	summer flounder	newly metamorphosed juvenile	14	7.23	4.49	5.70		
Paralichthys dentatus	summer flounder	newly metamorphosed juvenile	10	4.4	1.8	2.81		
Homarus americanus	American lobster	larval stage 2 to 3	4	5.4	3.9	4.59	4.47	3
Homarus americanus	American lobster	larval stage 2 to 3	4	5.0	3.7	4.30		
Homarus americanus	American lobster	larval stage 3 to 4	4	7.7	5.45	6.48		
Homarus americanus	American lobster	larval stage 3 to 4	4	4.9	3.8	4.32		
Homarus americanus	American lobster	larval stage 3 to 4	6	5.25	4.22	4.71		
Homarus americanus	American lobster	post larval stage 4 to 5	20	7.51	3.45	5.09		
Homarus americanus	American lobster	juvenile stage 5 to 6	27	3.50	1.53	2.31		
Homarus americanus	American lobster	juvenile stage 5 to 6	29	7.61	3.54	5.19		
Dyspanopeus sayi	Say mud crab	<48 hr old	8	6.81	4.21	5.35	4.61	2
Dyspanopeus sayi	Say mud crab	larval stage 1 to 3	7	3.31	2.45	2.85		
Dyspanopeus sayi	Say mud crab	larval stage 1 to 3	7	7.65	3.39	5.09		
Dyspanopeus sayi	Say mud crab	larval stage 1 to 3	7	4.46	3.51	3.96		
Dyspanopeus sayi	Say mud crab	larval stage 3 to 4	7	6.27	5.00	5.60		
Dyspanopeus sayi	Say mud crab	larval stage 3 to megalopa	4	5.44	4.40	4.89		
Dyspanopeus sayi	Say mud crab	larval stage 3 to megalopa	10	5.47	4.40	4.91		
Dyspanopeus sayi	Say mud crab	larval stage 3 to megalopa	11	7.54	3.23	4.93		
Labinia dubia	longnose spider crab	laval stage 1 to 2	7	5.30	4.11	4.67	4.67	1

[a]NOEC= no observed effect concentration; HOEC=highest observed effect concentration.

[b]Ranked by geometric means

growth, however, was not statistically significant. There was essentially no growth of surviving *M. menidia* at a DO concentration of 2.8 mg/L. Only the growth data were summarized in Table 2.

The second reason for restricting sub-lethal effects to growth is that results are available from only one saltwater test that measured reproductive effects. Data are presented in Appendix C from a 28-day life cycle test using the mysid *Americamysis bahia.* Although growth was reduced 25% at 3.17 mg/L and was technically the most sensitive endpoint in this test, the percentage reduction in growth was essentially the same at 2.76 and 2.17 mg/L as it was at 3.17 mg/L (20% and 27%, respectively). Reproduction was reduced by 76% at 2.17 mg/L, the first treatment that resulted in a significant effect on this endpoint. Although this test suggests that growth is more sensitive than reproduction, there are insufficient data to confirm this conclusion for saltwater species. Data from two standardized freshwater tests, however, indicate that growth is more sensitive than reproduction for both fathead minnows (Brungs, 1971) and *Daphnia magna* (Homer and Waller, 1983). Thus, DO limits that protect against growth effects also may be protective for reproductive effects.

Data on the affects of hypoxia on growth are presented for four species of fish and seven species of invertebrates from a total of 36 tests. The sensitivity of growth to low DO has been determined in only two standard 28-day tests which meet *Guidelines* requirements; the above life cycle test with *A. bahia* and the above early life stage test with *M. menidia.* Therefore, growth data from non-standard tests (i.e., not life cycle, partial life cycle or early life stage tests) were used to augment the chronic database. These non-standard tests ranged from 4 to 29 days long. Data from short duration tests were included because effects of oxygen deprivation are assumed to be instantaneous. Oxygen is required continuously for the efficient production of cellular energy. Therefore, even modest reductions in DO may result in the redirection of energy use from growth to compensatory mechanisms. In addition, data from larval growth of two bivalves (Morrison, 1971; Wang and Widdows, 1991) and several fish and crustaceans (Appendix C) show that chronic values for DO do not change substantially for exposures ranging from a few days to several weeks for most of the species tested. The *Mercenaria mercenaria* (Morrison, 1981) and *Mytilis edulis* (Wang and Widdows, 1991) studies show that the effect on larval bivalve growth within the same test run is the same over a series of days (13 days for *M. mercenaria* and 6 to 10 days for *M. edulis*).

Overall Genus Mean Chronic Values (GMCVs) for effects on growth range from > 1.97 for the sheepshead minnow *Cyprinodon variegatus* to 4.67 mg/L for the longnose spider crab *Labinia dubia*; a ratio of < 2.4. Three of the most sensitive species were crustaceans (Figure 3; Table 2). The range of chronic values for the four most sensitive genera is 3.97 to 4.67 mg/L; a ratio of only 1.2. The Final Chronic Value (FCV) was calculated in the same manner as the FAV (Stephan, et al., 1985). Because acutely resistant taxa are under-represented in the chronic database in Table 2, it could be argued that n, the number of genera used in the calculation of the FCV, should be increased from 11 to a higher value. We chose to increase n from 11 to 22 (the n for the FAV). This is the same procedure that was used for the FCV in the ambient water quality criteria for cad-

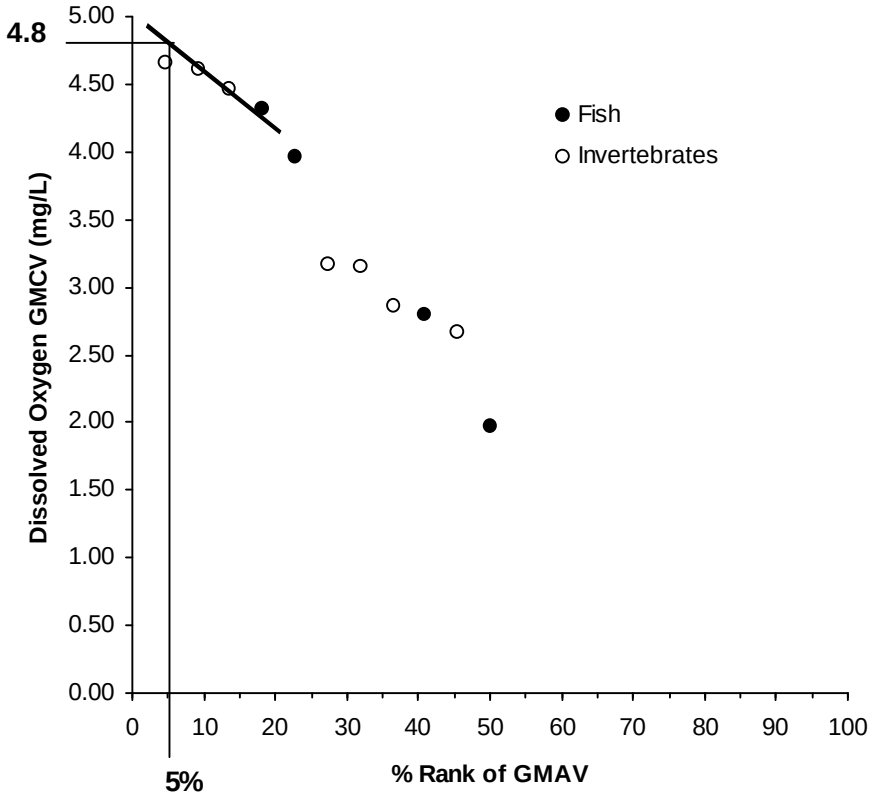


Figure 3. Plot of low dissolved oxygen effect (Genus Mean Chronic Values for growth) against percentile rank of each value in the data set. Percentile rank was adjusted based on the total "n" from the acute data set (see text for explanation). Specific values for each genus included are listed in Table 2. Results from individual tests for each species are listed in Appendix C. The value highlighted on the y-axis is the calculated Final Chronic Value (FCV). This value is the chronic value that is higher than the values for 95% of the species represented. The line drawn through the four most sensitive values is the line of best fit for those four values. The chronic values for the four most sensitive genera are the only values used in the FCV calculation other than the total number ('n") of values.

mium[7] (U.S. EPA, 1985). The final protective value for growth (the Criterion Continuous Concentration or CCC) is 4.8 mg DO per liter, but would increase only to 5.0 mg/L if n was kept at 11.

As presented here, the CCC is intended as a time-independent value. Areas where the average minimum DO does not fall below 4.8 mg/L should have sufficient DO to support the survival and growth of most aquatic species in the Virginian Province. Although it is generally accepted that reduced growth means reduced overall fitness, there is

[7] One assumption underlying the calculation procedure for FAVs and FCVs is that the sample of values available is representative of the population of values in the community being protected. If the dataset is too heavily weighted with values from the sensitive end of the distribution, then this skews the interpretation of the 5th percentile value that is calculated.

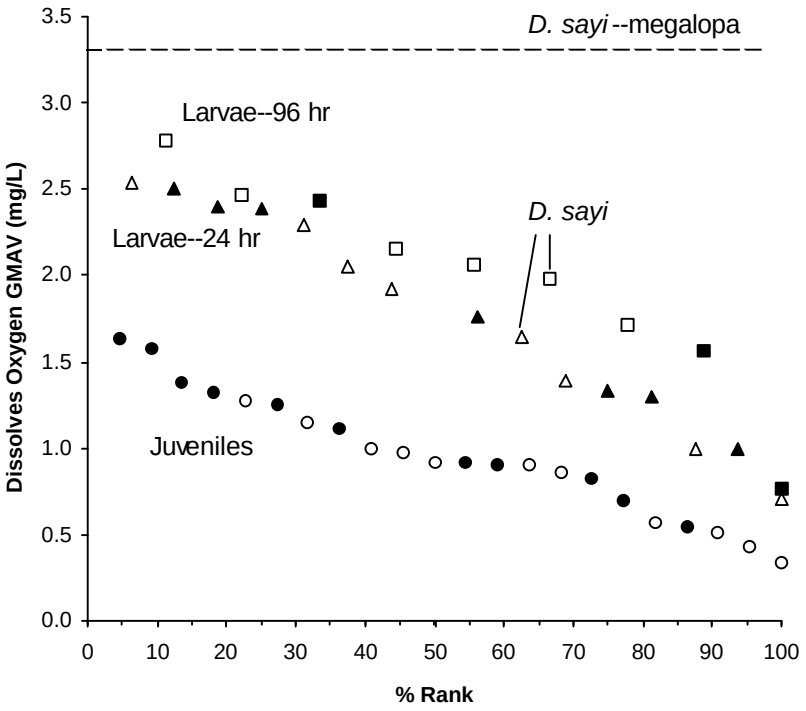


Figure 4. Plot of the GMAV data from Figure 2 (circles) along with 24 hr (triangles) and 96 hr (squares) LC50 values for larval life stages of various saltwater animals. The open symbols are for invertebrates and the closed for fish. The open square and triangle for *D. sayi* represent the mean response for all larval life stages for this species. The dashed line at top represents the LC50 for *D. sayi* exposed during the transition to megalopa. The data for the juveniles are from Table 1. The data for the larvae are listed in Appendix D.

little direct evidence for this in the field. In one study, Gleason and Bengtson (1996a,b) found that for some estuarine fish bigger is not necessarily better. Bigger fish (as prey) may be more susceptible to being eaten by predators. As an alternative to the growth criterion, a criterion that addresses chronic stresses from long-term or short-term exposures to low DC can be based on larval recruitment effects.

Larval Recruitment Effects

A generic model has been developed that evaluates the cumulative effects of acute and chronic stresses on early life stages of aquatic organisms. Early life history information and exposure-response relationships are integrated with duration and intensity of exposure to provide an ecologically relevant measure of larval recruitment. There are existing recruitment models for marine organisms (e.g., Ricker, 1954; Beverton and Holt, 1957). However, these models address other processes such as parental stock size, population fecundity, and density dependent processes such as cannibalism and intraspecific competition. These existing models therefore are not appropriate for the needs of the DO document, which requires incorporation of abiotic stressor effects.

Larvae are more acutely sensitive to low DO than juveniles (Figure 4); however, the criteria are not being established to protect larvae and juveniles in the same manner. A method is needed that estimates how many days a given DO concentration can be tolerated without causing unacceptable effects on total larval survival for the entire recruitment season. This is accomplished with a generic larval recruitment[8] model and applying biological and hypoxic effects parameters for the Say mud crab (*D. sayi*). Parameters for this larval crustacean are used for several reasons. Larval crustaceans are among the most acutely and chronically sensitive larval saltwater animals, and the Say mud crab's late larval to megalopa period is the most sensitive of the tested crustaceans (Table 2 and Figure 4). Among larvae at risk in estuaries, considerable information is available on Say mud crab with respect to the biological parameters in the model. Laboratory responses of *D. sayi* are indicative of a species the most at risk from hypoxia because it has a high DO response threshold. In addition, these larvae are present in the lower water column coincident with the expected hypoxia season present throughout the Virginian Province in salinities >15 ppt, which strengthens the choice of this species for a Province-wide model.

The model and the major assumptions used during its development are presented in Appendix E. The life history parameters in the model include only those that relate specifically to larvae: larval development time, larval season, attrition rate and vertical distribution. The recruitment model assumes that the period of low DO occurs within the larval season. The magnitude of effects on recruitment, defined as the cumulative number of successful transitions to megalopae, is influenced by each of the four life history parameters. For instance, larval development time establishes the number of cohorts that entirely or partially co-occur with the interval of low DO stress. The second parameter, the length of the larval season, is a function of the spawning period, and also influences the relative number of cohorts which fall within the window of hypoxic stress. The third life history variable, natural attrition rate, gages the impact of slower growth and development of the larvae in response to low DO by tracking the associated increase in natural mortality (e.g., predation). The model assumes a constant rate of attrition, so increased residence time in the water column due to delayed development translates directly to decreased recruitment. Finally, the vertical distribution of larvae in the water column determines the percentage of larvae that would be exposed to reduced DO under stratified conditions. Three exposure response curves that describe megalopa survival, zoea larval survival, and molt delay versus DO concentration are used for estimating recruitment under hypoxic conditions[9]. The model makes a simplifying assumption that hypoxic days are contiguous. The model can be applied either to establish protective conditions or to evaluate the severity of a given hypoxic condition.

The dose-response data used in the model in this document are presented in Figure 5. Figure 5A is a summary response curve for exposures that included a transition from zoea to megalopa. These tests were necessarily longer (7 to 11 days) than other larval tests to allow sufficient time for development to megalopa. Although some of the en-

[8] Once the larvae are "recruited" into the juvenile life stage, the juvenile protective limit established above is applied.

[9] The model is designed to allow both biological and exposure-response data to be changed based on the availability of appropriate data.

hanced sensitivity in these tests may be due to the longer exposures to low DO, mortality also appeared to be associated with the molt to megalopa[10]. The model assumes a constant rate of reproductive output per day, and a constant rate of development during the larval season. Therefore, some larvae in the plankton would be molting to this stage daily, and it is at this point that the crab larvae may be particularly sensitive to low DO. The model assumes that the response of the late larvae in transition to megalopae could occur following a single day of exposure (i.e., this response is independent of exposure prior to the day of transition). Thus, the model applies this dose response as a 24 hr exposure.

Figure 5B is a summary response curve for 24 hr exposures of zoea stage larvae. Figure 5C shows data that suggest a delay in development time for *D. sayi* in going from a stage 3 zoea to megalopa. However, the degree of developmental delay was difficult to measure with sufficient resolution. Further, it was difficult to distinguish it from differential survival sensitivity among individuals within a replicate. Thus, the model has been run with and without a delayed development effect. The results of these two runs are shown in Figure 6. Points on the graph show which combinations of low DO concentration and exposure duration result in a seasonal reduction of recruitment that does not exceed 5%. Until further information is available, the output used to establish the criteria for larval recruitment will be the one that assumes no delayed development (the solid line in Figure 6).

The equation for the larval line (as well as the lines in Figure 5A and 5B) was derived by an iterative process of fitting the best line through the points generated by the output of the recruitment model. The equation is a standard mathematical expression for inhibited growth (logistic function—Bittinger and Morrel, 1993). This equation is:

$$P(t) = \frac{P_0 L}{P_0 + e^{-Lkt}(L - P_0)} \qquad \textit{Equation 1}$$

For Figure 6, P(t) is the DO concentration at time t, P_0 is the y-intercept, and L is the upper DO limit. L was set as the DO concentration that allowed a 44-day exposure (the maximum exposure period the model allowed using the current parameters—see appendix page E-3 for further explanation). P_0 was first estimated by eye from the original plot, and then adjusted higher or lower to minimize the residuals between the real recruitment data and that estimated from the mathematical fit of the data. The rate constant, k, was similarly empirically derived. For Figures 5A and 5B, the variables t and L represent DO concentration and the upper limit for survival (100%), respectively.

[10] Data for another crustacean, *Cancer irroratus* (rock crab), also lend some support for having separate dose response curves for the zoea and megalopa larval life stages (Appendix F).

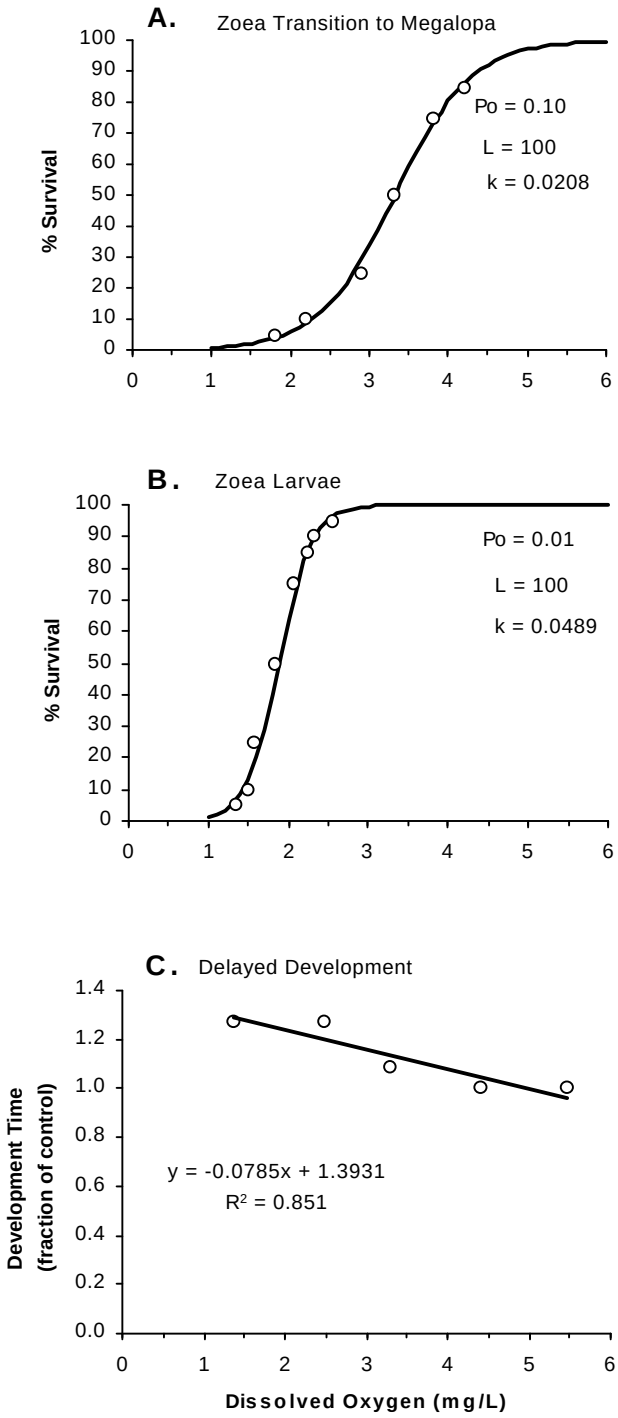


Figure 5. Dose response curves for Say mud crab (*Dyspanopeus sayi*) used in the larval recruitment model. Open symbols are the data from tests with continuous low dissolved oxygen exposures. Solid lines are the regression lines of best fit. See text for explanation of Po, L and k. A: dose response curve for zoea transition to megalopa. These data are from exposures durations greater than 24 hr but are applied as 24 hr exposures in the model (see text for explanation). B: dose response curve for zoea larvae. Data are from 24 hr exposures. C: data for delayed development of larvae to megalopae

Application of Persistent Exposure Criteria

The final criteria for saltwater animals in the Virginian Province (Cape Cod to Cape Hatteras) are indicated in Figure 7 for the case of continuous (i.e., persistent) exposure to low dissolved oxygen. The most uncertainty with the application of these limits usually will be when DO conditions are between the juvenile survival and larval growth limits. Below the juvenile survival limit, DO conditions do not meet protective goals. Above the growth limit, conditions are likely to be sufficient to protect most aquatic life and its uses. Interpretation of acceptable hypoxic conditions when the DO values are between the juvenile survival and larval growth limits depends on the characterization of the duration of the hypoxia. To determine whether a given site has a low DO problem, adequate monitoring data are required. The more frequently DO is measured the better will be the estimate of biological effects.

Figure 8 is a hypothetical time series for average daily dissolved oxygen minima. The portion of the data below the CCC is all that is considered. This area of the graph is first divided into several intervals. We recommend using no finer than 0.5 mg/L DO intervals because of limitations on most monitoring programs (see Implementation section). However, larger intervals may be necessary if monitoring data are not taken frequently enough. The resulting intervals in our example are (a) below 4.8 mg/L and above 4.3 mg/L, (b) below 4.3 and above 3.8, and so forth for intervals 'c' and 'd'. For each interval, the number of days is recorded that the DO is between the interval's limits. For example, in interval 'a' the DO is below 4.8 mg/L and above 4.3 mg/L from July 13th through the 18th and again from July 23rd through the 25th, for a total of seven days. This number of days is then expressed as a fraction of the total number of days that would be allowed for the DO minimum for each interval. For interval 'a', the allowed number of days is 24 (using Figure 6 at 4.3 mg/L). Table 3 lists the information for all four intervals from this hypothetical time series. The fractions of allowed days are totaled. If the sum is greater than one, then the DO conditions do not meet the desired protective goal for larval survival. If the sum is less than one (as is the case in our example), then the protective goal has been met. This procedure uses a simplifying assumption that each interval is independent. That is, there is no increased risk to recruitment due to pre-exposure to hypoxia. This assumption is supported by the similarity of larval survival data for 24 and 96 hr exposures in Appendix A.

The current recruitment model is a first attempt at providing a method that incorporates duration of exposure in the derivation of DO criteria. A model that could integrate gradual change in daily DO concentrations is desirable. However, the current model may be adequate given the probable inaccuracies in assessments of DO conditions in coastal waters (Summers, et al., 1997).

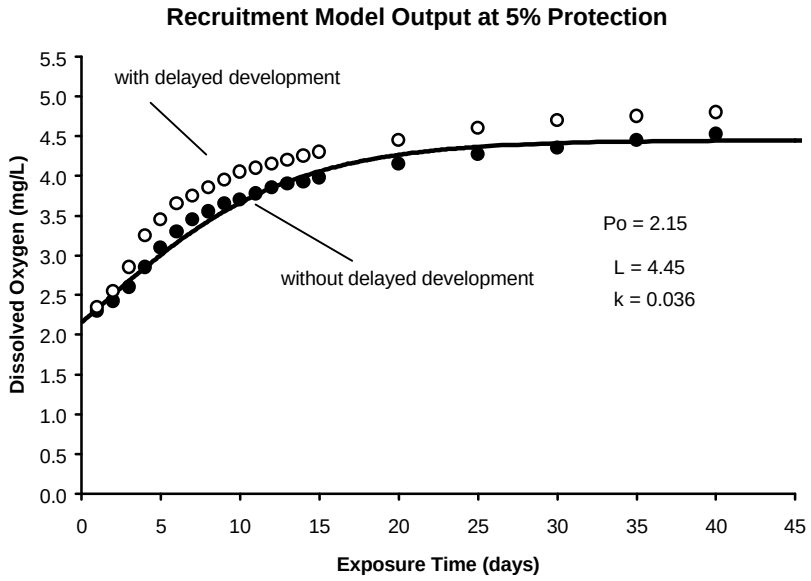


Figure 6. Plot of model output that protects against greater than 5% cumulative impairment of recruitment. Input parameters were the same for two runs of the model, except for the inclusion of the delayed development response (Figure 5C), open symbols, or the exclusion of molt delay, closed symbols, The solid line is the regression line of best fit for the closed symbols**.** The area below the line represents conditions of potential impairment. See text for explanation of Po, L and k.

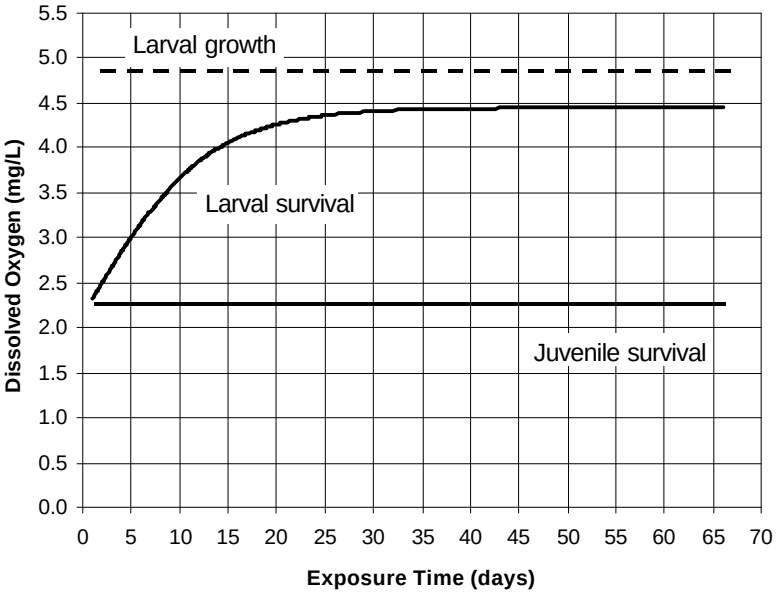


Figure 7. Plot of the final criteria for saltwater animals continuously exposed to low dissolved oxygen. The upper dashed line is the CCC for growth. The lower line is the CMC for juvenile (and adult) survival, and the curve between the two is the output from the recruitment model representing protective for larval survival. All of the lines are truncated at one day. The cyclic portion of the criteria addresses exposure less than 24 hr.

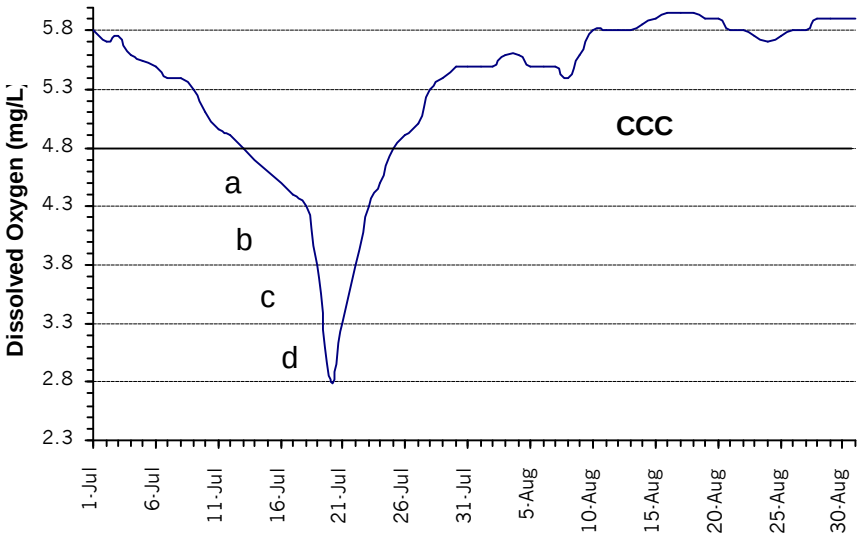


Figure 8. A hypothetical representative dissolved oxygen time series for one site. The horizontal line represents the CCC of 4.8 mg/L. The portion of the curve below 4.8 mg/L is divided into four arbitrary intervals (a,b,c,d) to estimate effects on larval recruitment. The dissolved oxygen minimum, and the duration for each interval are

Table 3. Dissolved oxygen and duration data from a hypothetical persistent time series (Figure 8). The Below and Above columns show the range of D.O. covered by each interval. Number of Days Within Range refers to the duration that the observed D.O. is between the range given. In the last column this duration is expressed as a fraction of the number of days allowed by the recruitment model (Figure 6) for the D.O. minimum of the interval. These fractions are totaled to evaluate whether the larval survival protective goal has been met.

	Range (mg/L)		No. Days	No. Days	Fraction of
Interval	Below	Above	Within Range	Allowed	Allowed
a	4.8	4.3	7	24	0.29
b	4.3	3.8	3	13	0.23
c	3.8	3.3	1	7	0.14
d	3.3	2.8	1	4	0.25
				TOTAL	**0.91**

Less Than 24 hr Episodic and Cyclic Exposure to Low Dissolved Oxygen

The criteria for continuous exposure to low dissolved oxygen do not cover exposures times less than 24 hr. This section addresses this topic by describing the available data and how they were used to evaluate the effect of low DO on exposure durations lasting less than 24 hr. These included one-time episodic events, as well as either tidal- or diel-influenced cycles where the DO concentrations cycle above and below the continuous CCC. The approaches described for treatment of non-constant (e.g., cyclic) conditions are intended to provide protective goals that are equivalent to those established for persistent conditions. The data used come from two types of experiments. The first are those which provide time-to-death (TTD) data and are used to derive TTD curves. The second are experiments in which there were treatments consisting of a constant exposure to a given low DO concentration paired with a treatment in which the DO concentration cycled between that low concentration and a concentration near saturation (or at least well above concentrations that should cause significant effects). The data from both of these experiments are discussed below.

Cyclic Juvenile and Adult Survival

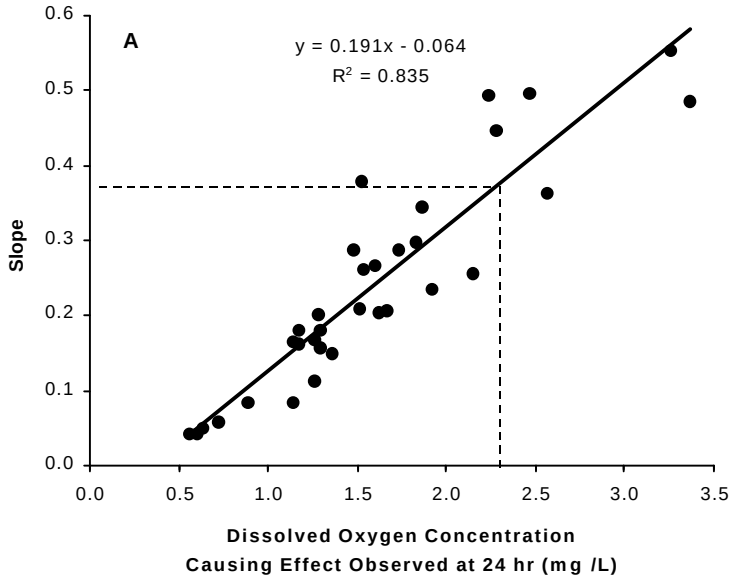
The persistent hypoxic criterion for juveniles and adults is 2.3 mg/L. A conservative estimate of the safe DO concentration for exposures less than 24 hr would be to simply use 2.3 mg/L. However, time-to-death data indicate that this would be over protective. Data are available for two saltwater juvenile fish (*Brevootia tyrannus* and *Leiostomus xanthurus*), one freshwater juvenile fish (*Salvelinus fontinalis*), and three larval saltwater crustaceans (*D. sayi, Palaemonetes vulgaris* and *Homarus americanus*), providing a total of 33 TTD curves (Appendix G). The curves represent a range of test conditions, including acclimation to hypoxia with *S. fontinalis,* and a range of lethal endpoints. Two general observations were made from this data. First, each curve can be modeled with the same mathematical expression, a logarithmic regression, of the form:

$$Y = m(\ln X)+b \qquad \textit{Equation 2.}$$

where X=time, Y=DO concentration, m=slope and b=intercept where the line crosses the Y-axis at X=1.

Second, the shape of the curve (i.e., the slope and intercept) was governed by the sensitivity of the endpoint. This is true whether the sensitivity increase was due to interspecific differences (including saltwater and freshwater species) or the use of different endpoint (e.g., LC5 is more sensitive endpoint than LC50).

Figure 9 shows the relationship between sensitivity (i.e., 24 hr LC values) and the slope (Figure 9A) and the intercept (Figure 9B) for all 33 TTD curves (Appendix G). The DO value from each TTD curve at 24 hr was used as a measure of sensitivity. Plots using other time intervals could have been used. The value at 24 hr was chosen in order to generate a curve for juveniles that meets the constant CMC at its 24 hr value (2.3 mg/L). The slope and intercept for a time-to-CMC curve were calculated using Figure 9 equations and the CMC 24 hr value of 2.3 mg/L. These were then used as the parameters in Equation 2 to generate a criterion for saltwater juvenile animals for exposures less than 24 hr (Figure 10).



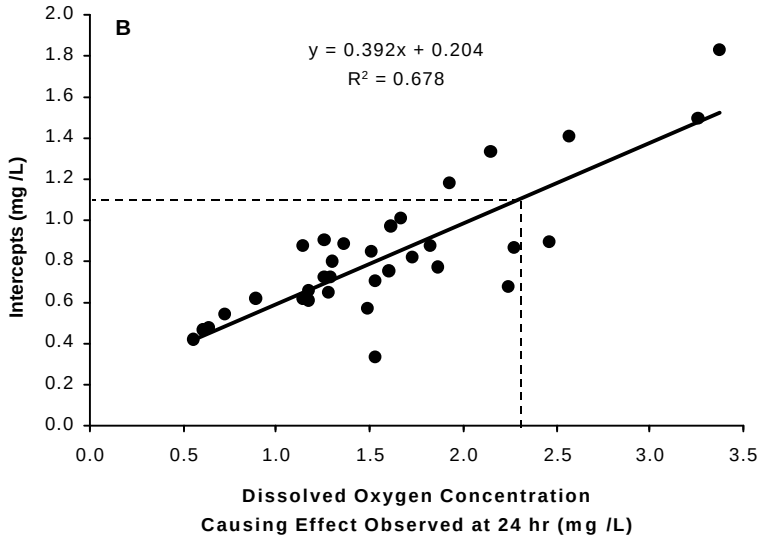


Figure 9. Slope (A) and intercept (B) versus low D.O. effect values at 24 hr from time-to-death (TTD) curves for two species of saltwater juvenile fish, one species of juvenile freshwater fish and three species of saltwater larval crustaceans. Data used mostly represent LT50 curves, but values for other mortality curves are included. Species used and their associated TTD curves are presented in Appendix G. All TTD curves were fit with a logarithmic regression.

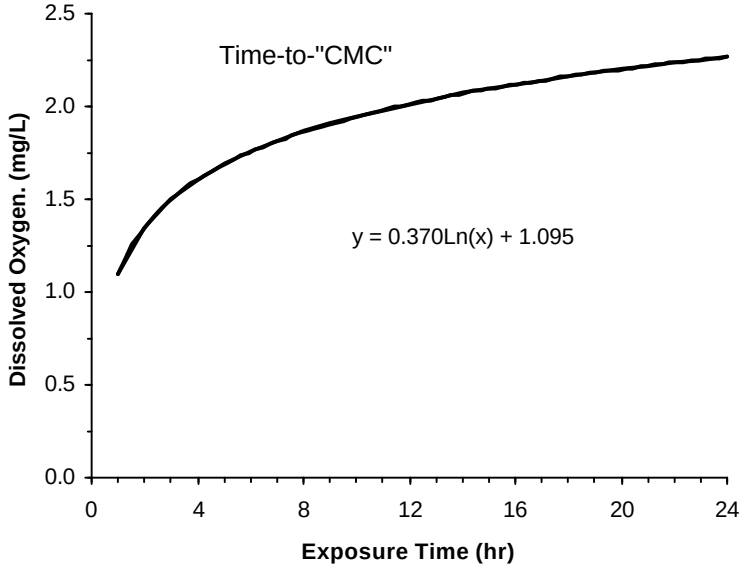


Figure 10. Criterion for juvenile saltwater animals exposed to low dissolved oxygen for 24 hr or less. The line represents the same protective limit as the CMC for juveniles for continuous exposure. The line is a logarithmic expression with a slope and intercept calculated from the regressions in Figure 9 at the dissolved oxygen concentration of 2.3 mg/L (the CMC).

Cyclic Growth Effects

The CCC for continuous exposure was derived based on growth effects data (Table 2). The simplest way to determine effects from cyclic exposure to low DO is to compare growth of organisms under cyclic conditions to those for the same species under continuous conditions. Growth data are available from cyclic exposures to low DO for three species of saltwater animals, *D. sayi*, *P. vulgaris* and *Paralichthys dentatus* (Coiro, et al., 1999). These data are listed in Appendix H and summarized in Figure 11. Data are from experiments in which a low DO treatment was paired with a treatment cycling between the same low DO concentration and one that was above the continuous CCC (usually saturation). All cyclic treatments had 12 hr of low DO within any one 24 hr period. Most of the cycles consisted of 6 hr at the low concentration followed by 6 hr at the high concentration. Only two tests (both with *P. vulgaris*) were conducted using a 12hr:12hr cycle. There were a total of 20 paired treatments spread among the three species.

As expected, at the end of each test, cyclic exposures generally resulted in more growth than constant exposures to the minimum DO of the cycle (Figure 11). However, if the effects of DO on growth were instantaneous (i.e., growth reduction begins as soon as the DO concentration drops and growth rate returns to normal as soon as DO returns to above CCC concentrations), then the cyclic exposures in the above experiments would have been expected to cause one half of the growth reduction observed in the constant treatment of each pair. (As noted above, the DO cycles had a total of 12 hr of low DO per day.) If this were true, then the slope of the line in Figure 11 would be 0.5. However, the slope of the line for the data (forced through the origin) is 0.778, a factor of 1.56 greater.

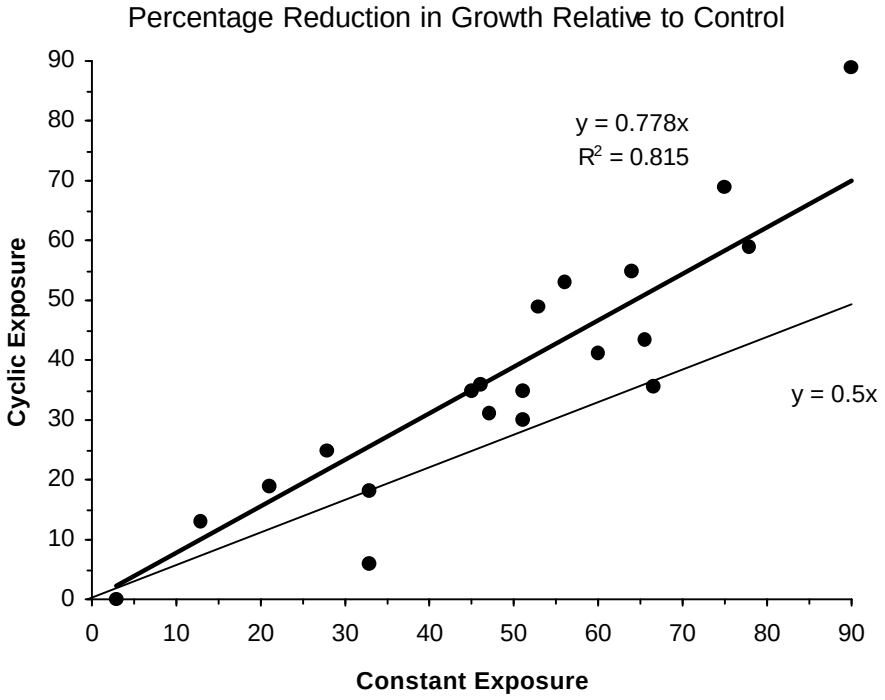


Figure 11. Plot of test results from growth experiments pairing constant low dissolved oxygen exposure with exposures to various cycles of low dissolved oxygen and concentrations above the CCC. The dark line is a linear regression of the data with the line forced through the origin. The lighter weight line is the "expected" relationship from a slope of 0.5 (see text for explanation). Species used and the experimental conditions are listed in Appendix H.

Thus greater growth impairment occurs from cyclic exposures than expected. One hypothesis for this discrepancy is that recovery from the low DO portion of the cycle is not instantaneous, and the actual low DO effect period is then greater than 12 hr within each day (by a factor of 1.56)[11].

Figure 12 shows a dose-response for growth of larval Say mud crab (*D. sayi*) over a range of constant DO concentrations[12]. The data are from ten tests (see Appendices C and H) with durations ranging from 4 to 11 days. The percentage growth reduction is relative to a control response. Growth reduction effects are considered instantaneous, therefore the % reduction can be applied to any time period. Data for this mud crab are emphasized because it was the only sensitive species tested in cyclic exposures. In addi-

[11] The data used to establish the relationship between cyclic and constant exposures (Figure 11) came from experiments with a total low DO exposure of 12 hr per 24 hr period. We assume that as the total time of exposure per 24 hr decreases the discrepancy between expected and observed should also decrease. Thus the 12-hr data can be considered a worst case for any daily cycle of 12 hr or less exposure to low DO. There is insufficient information for cycles with greater than 12 hr exposure periods per day. We recommend assuming constant exposure conditions for these latter situations.

[12] The relative sensitivity of Say mud crab growth to low DO versus other species tested is shown in Appendix I.

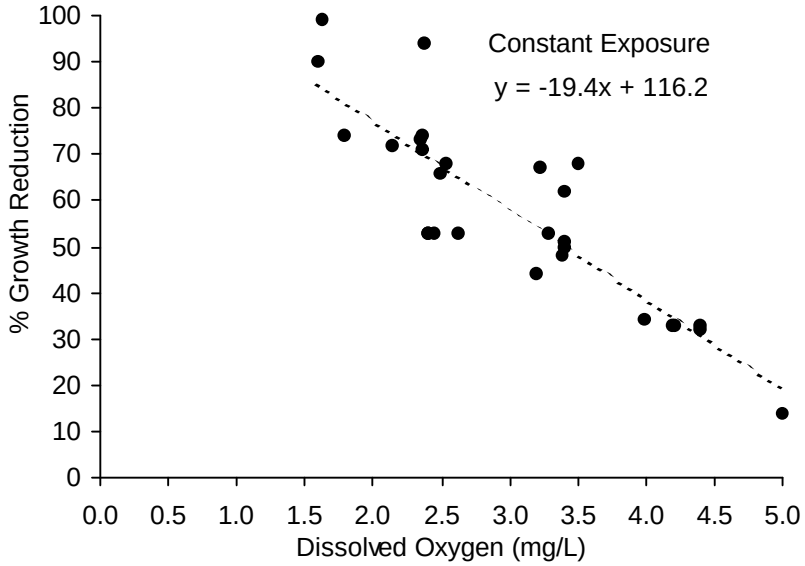


Figure 12. Plot of dose-response data for growth reduction in Say mud crab (*Dyspanopeus sayi*) exposed to various continuous low dissolved oxygen concentrations. Percentage growth reduction is relative to a control. The dashed line is a linear regression through the data points.

tion, this species is used to represent larval crustaceans in the recruitment model for constant exposures.

To evaluate a cycle for chronic growth effects, the above relationship between cyclic and constant exposure is needed as well as monitoring data from a representative, or worst case, cycle of low DO for a given site. Figure 13 provides a hypothetical DO time series. To estimate the expected growth reduction during this cycle the curve is divided into three DO intervals[13] for that portion of the cycle that falls below 4.8 mg/L (the CCC). The DO mean, and the total duration that the cycle is within the interval's range of DO, are determined for each interval. Data from this example are presented in Table 4. Interval 'c' lasts a total of five hours. Interval 'b' lasts a total of three hours (b1 before plus b2 after interval 'c'). Similarly, interval 'a' lasts for a total of four and a half hours. Each of these time intervals is multiplied by 1.56 to adjust for the cyclic effect.

A DO mean concentration for each interval is used with the equation from Figure 12 to estimate a daily growth reduction that is expected for larval crustaceans during constant exposure to hypoxia. This value is then normalized for the interval's cyclic adjusted duration. The normalized reductions for all intervals are added (growth effects are cumulative) for an estimated growth reduction for the cycle. This reduction is compared to the reduction estimated to occur at the CCC for constant exposures (23%, using the

[13]Any number of intervals can be chosen, even one. For simplicity, different DO ranges can be selected for each interval so that each interval has approximately the same total time below the CCC. Alternatively, the cycle can be divided by selecting a constant DO range (e.g., 0.5 mg/L), giving each interval a different time value. Monitoring data, however, must be frequent enough to justify the chosen interval size.

equation from Figure 12 at 4.8 mg/L DO). The percentage reduction in our example is 34%. This reduction is greater than the maximum allowed by the CCC, thus our hypothetical cyclic hypoxic event does not meet the protective goal for growth.

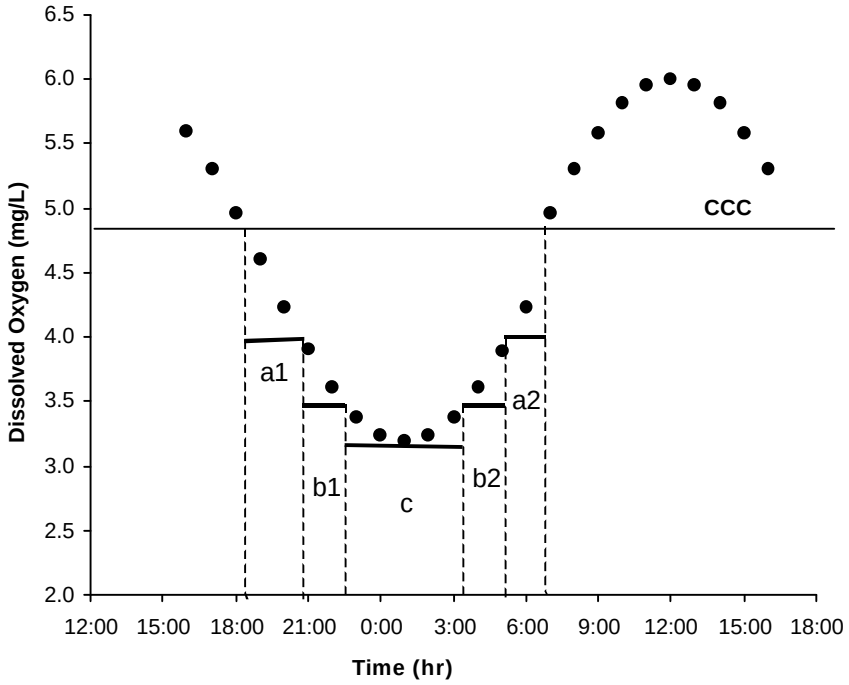


Figure 13. A hypothetical representative dissolved oxygen time series for one cycle. The horizontal line represents the CCC of 4.8 mg/L. The portion of the curve below 4.8 mg/L is divided into three arbitrary intervals (a,b,c) to estimate effects on growth. The range of dissolved oxygen, the mean dissolved oxygen and the duration for each interval are listed in Table 4.

Table 4. Dissolved oxygen and duration data from a hypothetical cyclic time series (Figure 13). These data are used to estimate the growth reduction occurring for the recruitment modeled species during the cycle. Percentage reductions in growth for constant exposure are calculated with the equation in Figure 12. These in turn are normalized for the cyclic adjusted duration.

Interval	D.O. Range (mg/L)	D.O. Mean (mg/L)	% Daily Reduction in Growth	Actual Duration (hr)	Cyclic Adjusted Duration (hr)	% Reduction for Duration
a1 – a2	4.8 – 4.0	4.40	31	4.5	7.0	9
b1 – b2	4.0 – 3.5	3.75	43	3	4.7	8
c	3.2 – 3.5	3.35	51	5	7.8	17
				Total % Reduction for Cycle		**34**

Cyclic Larval Recruitment Effects

In order to evaluate cyclic exposures for their potential impact on larval recruitment to the juvenile life stage two pieces of information are needed. First, a set of larval crustacean time-to-death curves to estimate the expected daily mortality for a given low DO cyclic exposure. Second, a way to translate that predicted daily larval mortality into allowable days for the given low DO cycle using the constant exposure recruitment model output. Creation of the larval TTD curves is straightforward using the sensitivity information (dose response curve) for the Say mud crab late larval to megalopa transition period in Figure 5A[14] and the sensitivity dependent relationships for TTD slopes and intercepts in Figure 9. Creation of a series of larval TTD curves followed the same procedure used to create the time-to-CMC curve for juveniles (Figure 10). Figure 14 shows the results for nine calculated curves for mortalities ranging from 5 to 95%.

Estimating the daily mortality expected to occur with the model species also is straightforward, and as with cyclic growth protection, requires representative or worst case DO monitoring data. Figure 15 is a hypothetical monitoring data set for a single cycle. As with growth, the portion of the cycle below the CCC is first divided into several intervals. The DO minimum is determined for each interval. It should not matter how the intervals are selected. All that is needed is a set of paired time and DO values. Table 5 lists the data for the intervals in this example. These data were plotted among the family of larval TTD curves (Figure 16). In the example, the greatest effect datum lies closest to the 10% mortality curve. Therefore, the hypothetical cycle of DO is expected to cause 10% daily mortality to the modeled larval crustacean. We are only concerned with the greatest effect datum because survival effects are not cumulative (i.e., an individual can only die once).

Now all that is needed is to translate the expected 10% mortality into the number of allowable days for this hypothetical cycle to occur. This is accomplished using the fitted curves in Figures 5A and 6. Figure 5A is the dose response curve for the Say mud crab late larval transition to megalopa period used in the recruitment model. The information in the figure is for percentage survival, but it can be converted easily into percentage mortality. Thus the information shows the expected cohort mortality to occur for a given DO concentration. For the example, 10% mortality occurs at a DO concentration of 4.4 mg/L. From the equation used to fit the data in Figure 6, the 4.4 mg/L is allowed to occur for up to 26 days without significant impairment to seasonal recruitment. Thus, the cycle that resulted in an estimated 10% daily mortality to larval crustaceans can be repeated for up to 26 consecutive days without exceeding a 5% reduction in seasonal larval recruitment to the megalopa life stage. All of the above can be simplified by merging the information from Figures 5A and 6 into one cyclic translator figure using the DO axis that is common between Figures 5A and 6. This is shown in Figure 17.

[14]The late larval to megalopa dose-response curve was selected because it is the most sensitive curve used in the recruitment model.

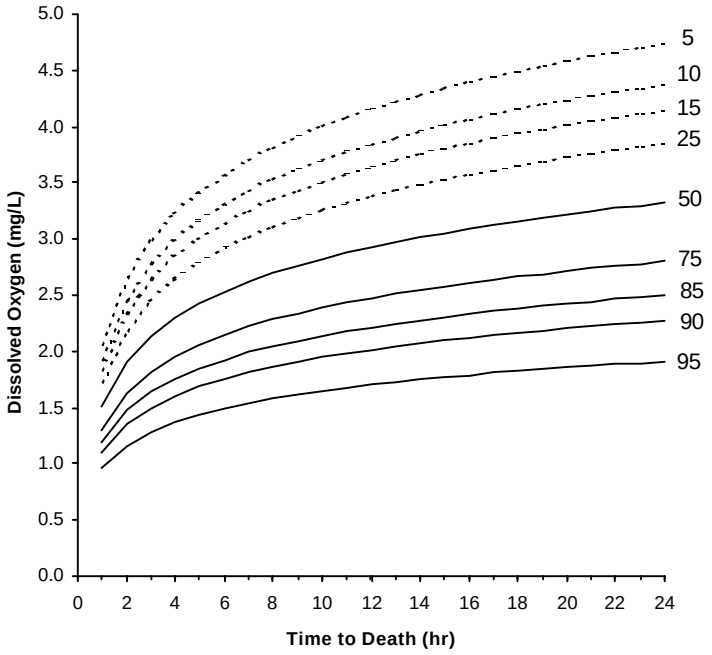


Figure 14. Time-to-death (TTD) curves generated for the recruitment model species. Data to generate the curves were taken from Figures 5A, 9A and 9B. The numbers adjacent to each TTD curve are the percentage mortality that each curve represents. The dashed lines represent curves created with slopes and intercepts outside the range of the original data used in Figure 9.

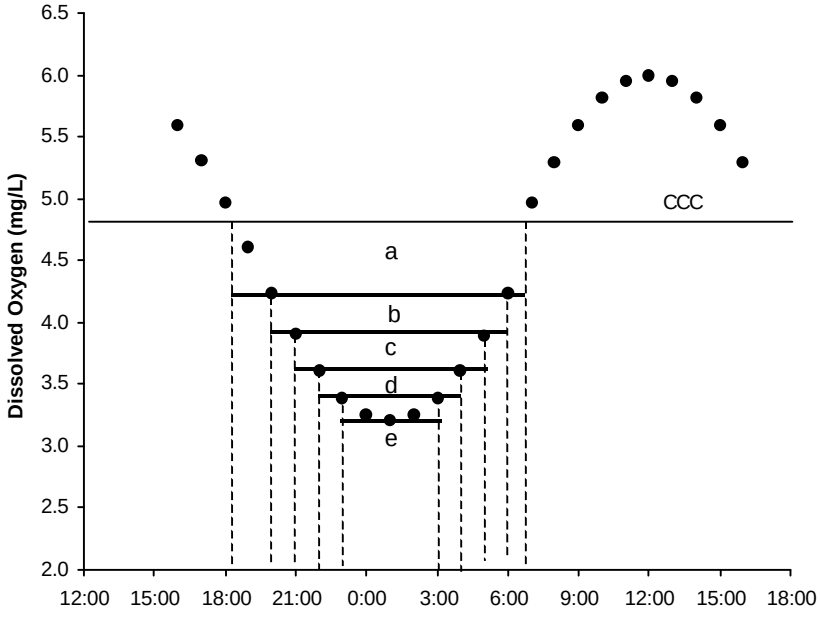


Figure 15. The same hypothetical dissolved oxygen time series as Figure 13. This time the portion of the curve below 4.8 mg/L is divided into several arbitrary intervals to estimate effects on mortality. The dissolved oxygen minimum and its duration for each interval are listed in Table 5.

Table 5. Dissolved oxygen and duration data from the intervals selected from the hypothetical cyclic time series in Figure 15. These data are plotted in Figure 16 to estimate the expected mortality occurring for recruitment modeled species during the cycle.

Interval	D.O. Minimum for Interval (mg/L)	Duration of Interval (hr)
a	4.2	12
b	3.9	10
c	3.6	8
d	3.4	6
e	3.2	4

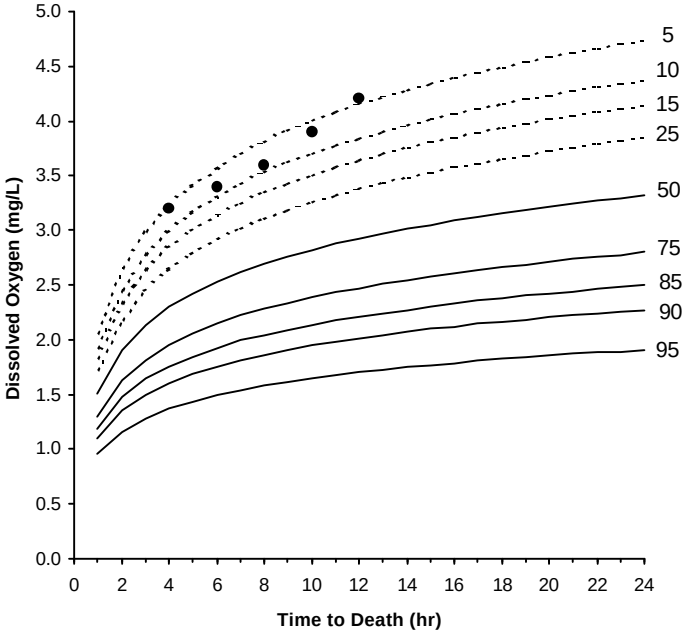


Figure 16. The dissolved oxygen minima and the durations listed in Table 5 superimposed on Figure 14 (solid circles). The expected mortality from the cyclic exposure is determined by the data point falling *closest to a TTD curve of greatest effect*, in this case 10% mortality.

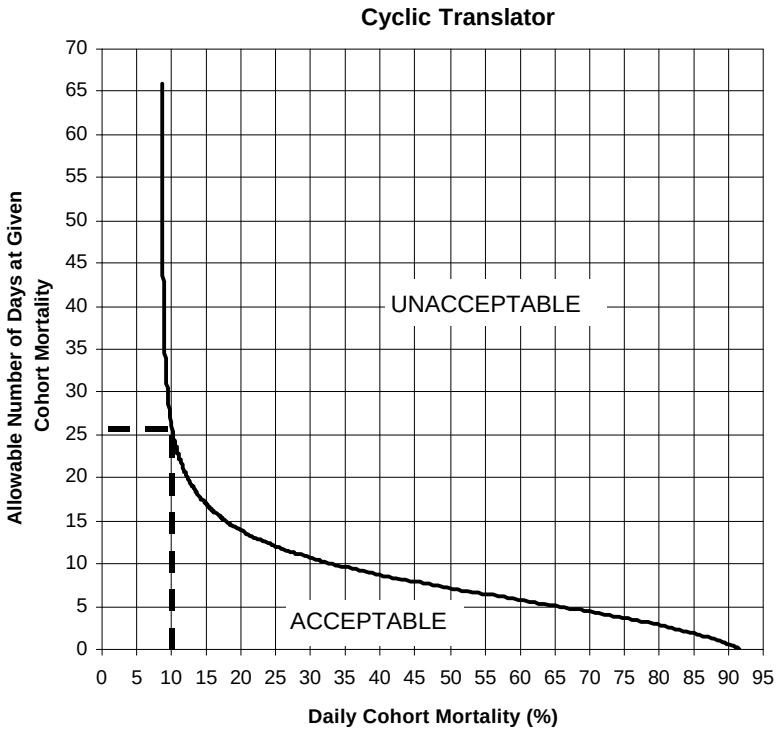


Figure 17. A plot that combines the information from Figures 5A and 6 into a single cyclic translator to convert expected daily mortality from cyclic exposures into allowable number of days of those cycles.

Other Laboratory Bioassay Data

Additional available data on lethal and sublethal effects of hypoxia on saltwater animals (Appendix J) do not indicate significantly greater sensitivity than indicated previously. The other data are divided into effects on juveniles and adults, and effects on larvae. Figure 18 shows all of the juvenile mortality data from Appendix J plotted against the criteria for juvenile and adult survival (limits for both persistent and cyclic exposures are included). Most of the other survival data are well below the criteria. There are three notable exceptions. The first is a single datum (LC50 of 1.9 mg/L) for the Atlantic menhaden *Brevoortia tyrannus* at 6 hr (Voyer and Hennekey, 1972). However, several other LC50 values (Burton et al., 1980) for Atlantic menhaden with durations ranging from 2 to 72 hr were much less (0.70 to 0.96 mg/L). The second is a single datum for the Atlantic silverside *M. menidia* at 6 hr (also Voyer and Hennekey, 1972). There are no other data for juvenile Atlantic silversides, but the unusually high sensitivities reported by Voyer and Hennekey for the other species suggest that their exposure system might be a confounding factor. In addition, the authors provided no information on control response for either the Atlantic menhaden or the Atlantic silversides.

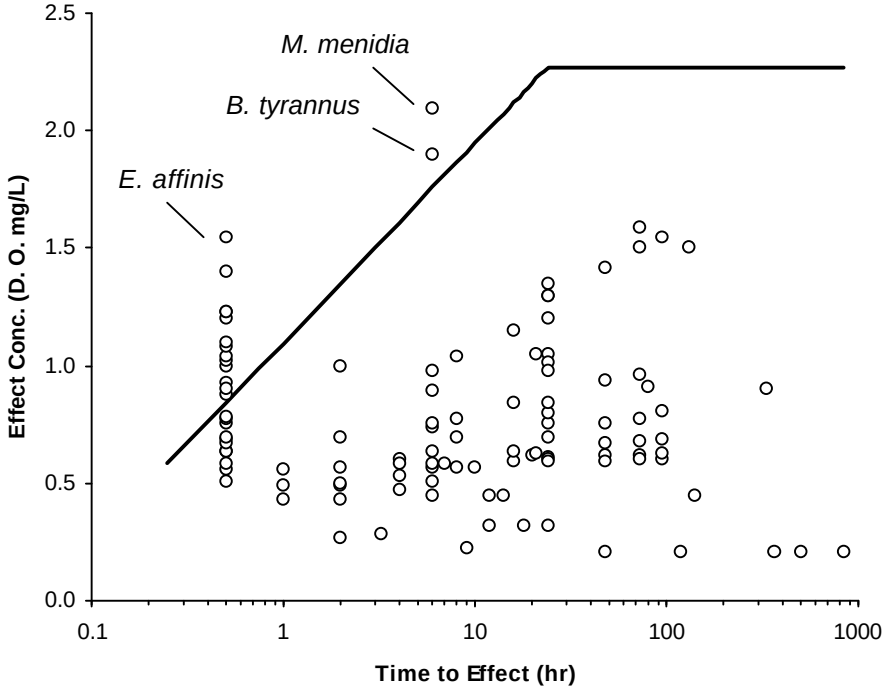


Figure 18. A plot of the other juvenile/adult mortality data from Appendix J (open symbols) along with the proposed dissolved oxygen criteria for juvenile/adult survival (solid line).

The third set of data above the criteria is a series of values at 0.5 hr for the copepod *Eurytemora affinis*. Some are below the criteria, but many are above it (Vargo and Sastry, 1978). However, the authors did not give any details on their experimental methods, including the number of replicates, the number of animals in each replicate, or on the response in the control. Thus, it is difficult to adequately access the significance of these results. However, in the absence of data to the contrary, it is worth noting the DO limit for juveniles and adults may not be protective of copepods. Alternatively, one could consider that short-lived species with high reproductive outputs (such as copepods) may be more appropriately protected in a manner similar to larval recruitment. In this case all of the *E. affinis* LC50 values would fall below the criterion provided by the larval recruitment (see explanation for Figure 19A below).

Figures 19A and 19B present all of the lethality data from Appendix J for tests using larval life stages. All of these data are from tests for effects on individuals, and the criterion for larval survival acknowledges that some larval mortality is acceptable. Most of the data for larvae are LC50 values for exposure durations other than 24 or 96 hr (these two durations are used elsewhere in the document). The LC50 data are plotted in Figure 19A. The most appropriate protective limit to compare these values with is the time-to-death (TTD) curve for 50% mortality for the Say mud crab (from Figure 14), because the larval survival protective limit is based on data for this species. There are two series of data points for LC50 values for larval rock crab *Cancer irroratus* for exposure durations of two and four hours; each has some values above the 50% TTD curve (Vargo and Sastry, 1977). The more sensitive values in these sets are for tests run at 25°C, thus the animals were likely exposed to multiple stressors (temperature and low DO).

The rest of the other lethality data for larvae are plotted in Figure 19B. These data are separated into three categories, LC5 toLC35, LC40 to LC65, and LC90 to LC100. As with the LC50 values in Figure 19B, these values are plotted along with time-to-death curves (10, 50 and 90% mortality) for late larval Say mud crab (From Figure 14). All of the LC5 to LC35 values are at or below the 10% TTD curve. All of the LC40 to LC65 values are well below the 50% TTD curve. Finally, all but one of the LC90 to LC100 values are below the 90% TTD curve. This one value is for 100% mortality of stripped bass larvae, *M. saxatilis that* occurred after a 2 hr exposure to 1.90 mg/L DO. However, there are two other stripped bass tests were 100% mortality of the larvae did not occur until 24 hr of exposure to similar low DO.

The are fewer other data on sublethal effects than for lethality effects (Appendix J). The sublethal effects included reduced feeding, growth, locomotion, and bivalve settlement, as well as delays in hatching and molting. However, none of these values indicate that the CCC would not be protective against these effects.

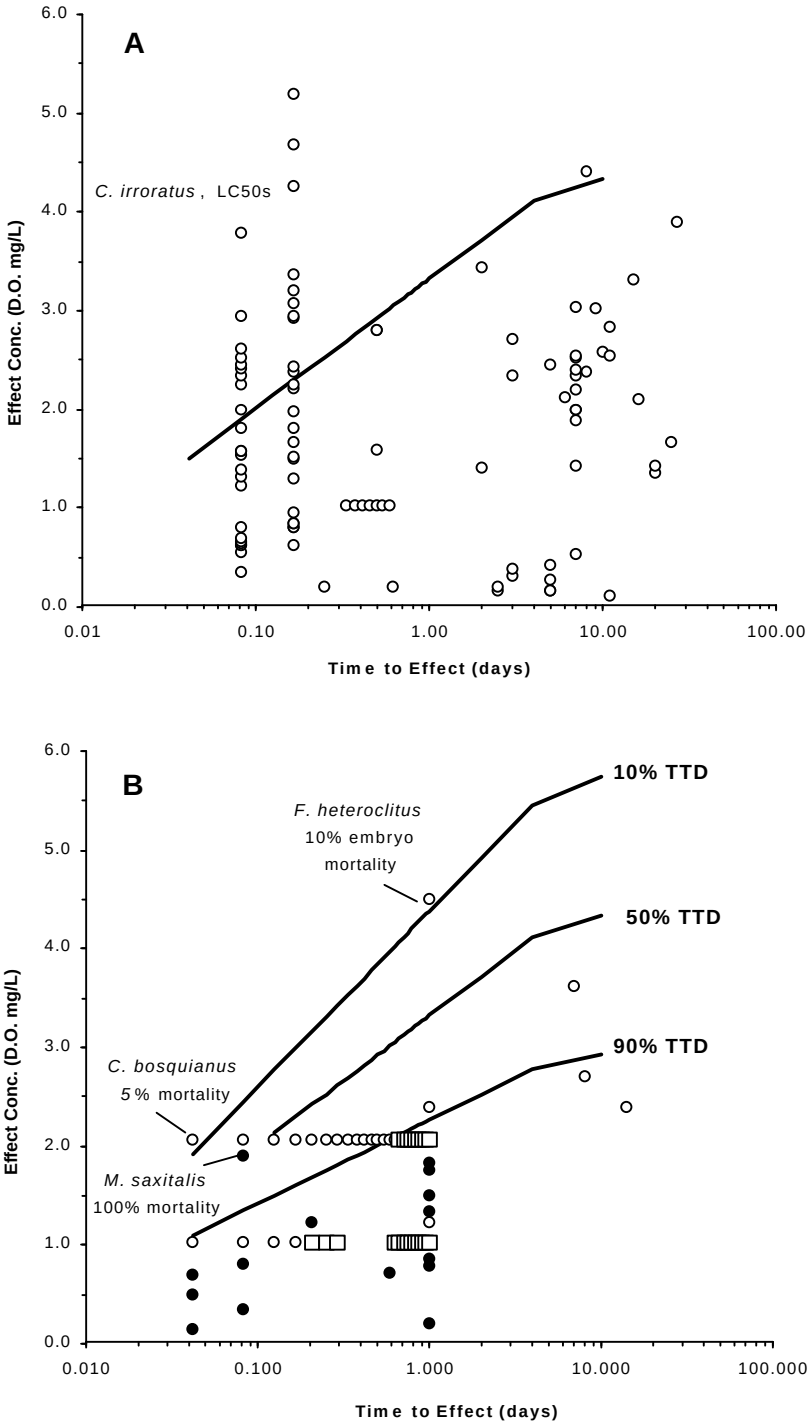


Figure 19. A plot of the other larval survival data from Appendix J. Figure 19A presents the available LC50 data (open circles) along with the 50% time-to-death (TTD) curve for the Say mud crab. Figure 19B presents mortality data for other than 50%. Open circles represent 5 to 35% mortality, open squares 40 to 65% mortality, and closed circles 90 to 100% mortality. Figure 19B also includes the 10, 50 and 90% TTD curves for the Say mud crab.

Laboratory Observed Behavioral Effects of Hypoxia

A number of laboratory studies report behavioral alterations following exposure to hypoxia. The effects include low DO avoidance, changes in locomotion, burrowing and feeding activity, and altered predator-prey behaviors. Most of the effects observed occurred <2.3 mg/L, hence would be protected by even the 24 hr acute limit CMC. The most hypoxia-sensitive behavioral effect occurs in red hake (*Urophycis chuss*). In red hake, age 0+ fish leave their preferred bottom habitat and begin to swim continuously as DO concentrations fall below 4.2 mg/L (Bejda et al., 1987). Food search time is also reduced as a consequence. Below 1.0 mg/L, most locomotor and other behavioral activity ceases, and at 0.4 mg/L there is loss of equilibrium. Older red hake, (age 1+ and 2-3+), did not exhibit these responses with low DO, except for loss of equilibrium at 0.6 mg/L.

The following effects are reported at less than the 2.3 mg/L protective limit. In the red morph of green crabs (*Carcinus maenas*) the low DO avoidance EC25 was <2.3 mg/L and the EC50 was 1.8 (Reid and Aldrich, 1989). The green morph was less sensitive. In naked goby (*Gobiosoma bosc*) larvae, avoidance at 2.0 mg/L occurred with $\geq$ one hr exposure (Breitburg, 1994). No avoidance was observed at 3.0 mg/L. This same author reported 100% avoidance in larval bay anchovy (*Anchoa mitchilli*) at 0.75 mg/L following a one hr exposure. Reduced locomotor activity occurred in daggerblade grass shrimp (*P. pugio*) at 1.8 mg/L (Hutcheson et al., 1985). Burrowing in the northern quahog (*M. mercenaria*) was reduced 1.4 to 2 fold when exposed to 1.8 to 0.8 mg/L and slowed 4 fold in Atlantic surfclam (*Spisula solidissima*) at 1.4 mg/L (Savage, 1976). The polychaete, *Nereis virens*, EC25 for emergence from the sediment was 0.9 mg/L (Vismann, 1990). The shelter guarding and nest guarding behavior by adult male naked goby (*G. bosc*) was not altered at 0.7 mg/L, but they abandoned shelters at 0.38 mg/L and nests at 0.3 mg/L. Death occurred in these animals at 0.26-0.24 mg/L (Breitburg, 1992).

The following low DO effects on feeding are reported in a bivalve and four polychaetes. In eastern oyster (*Crassostrea virginica*) early post-settlement stage (436 µm mean shell height), exposure to 1.9 mg/L for 6 hr resulted in 54 to 61% reduction in feeding rate; at <0.4 mg/L for the same period, 86 to 99 % reduction occurred (Baker and Mann, 1994b). In older post-settlement animals (651 µm mean shell height), feeding rate was not altered with 1.9 mg/L exposure for 6 hr, but at < 0.4 mg/L it was reduced 97 to 99%. In the polychaetes, feeding stopped in *Nereis diversicolor* at 1.2 mg/L and in *N. virens* at 0.9 mg/L (Vismann, 1990). In adult *Loimia medusa*, feeding stopped at 1.0 mg/L during <20 hr exposure, then resumed in 42 to 113 hr in 42% of the animals (Llansó and Diaz, 1994). At 0.5 mg/L, there was no resumption of feeding after initially ceasing during the same initial exposure period. Following exposure in *Streblospio benedicti* adults, the initial response to 1.0 mg/L was cessation of feeding, but it resumed in 3.5 days; with 0.5 mg/L exposure, the initial response was the same, with feeding resuming in 4.5 days (Llansó, 1991).

Changes were observed in predator-prey activities in two fishes in low DO In naked goby (*G. bosc*) larvae, avoidance of the sea nettle (*Chyrsaora quinquecirrha*) predator was reduced 60% following 3 hr exposure to 2.0 mg/L. In striped bass (*M. saxatilis*) juveniles, predation on naked goby larvae was reduced 50% following 1 hr 35 min exposure to 2.0 mg/L (Breitburg et al., 1994).

Observed Field Effects

Field reports of the biological consequences of hypoxia could be used to derive DO criteria if they include information to describe the exposure conditions. Yet sufficient data are rarely available. In most cases, DO conditions prior to observed effects are unknown, making it difficult to predict an exposure threshold for the observed effect. A field report of hypoxic effects must, at a minimum, provide a description of the concurrent DO exposure conditions if it is to be useful in deriving criteria. Ten studies in the Virginian Province have provided concurrent DO measurements. The DO observations often are only point measurements, not continuous records, and they rarely provide information on DO conditions prior to the observed effects. The biological effects reported include alterations in the following: presence of fish and crustaceans, diel vertical migration of copepods, recruitment and population density of an oyster reef fish (naked goby), recruitment and growth of eastern oyster spat, and macrobenthic community parameters. Effects were usually not observed above 2 mg/L. Exceptions are the Long Island Sound trawl studies, where effects were reported in the 2.0 to 3.7 mg/L range.

The relationship between low DO and presence of fish and shellfish in Long Island Sound was examined in two trawl studies. Howell and Simpson (1994) reported marked declines in abundance and diversity in 15 of 18 study species when DO was below 2 mg/L. When DO was between 2 and 3 mg/L, there were significantly reduced abundances of three species: winter flounder, windowpane flounder and butterfish. In a subsequent three year study, the aggregate data for 23 species of demersal finfish showed a decline for two community indices, total biomass and species richness, with declining DO (Simpson et al., 1995). The DO concentration that corresponded with a 5% decline below a response asymptote was 3.7 mg/L for total biomass and 3.5 mg/L for species richness. Dissolved oxygen declines below these concentrations resulted in further exclusion of these animals, which has implications for the secondary productivity of these waters. Reduced species number implies reduction of community resilience, should this condition persist. The consequences of habitat crowding on animals occurring in adjacent waters is unknown.

Hypoxia-induced changes in the distribution of fish and crustaceans have also been reported in the lower York River, located in the Virginian portion of Chesapeake Bay (Pihl et al., 1991). Subpycnocline DO <2 mg/L developed during neap tide periods and the study species (spot, croaker, hogchoker, blue crab, and mantis shrimp) migrated to shallower and better oxygenated habitats. The degree and order of vertical movement was believed to be a function of the water column DO concentration and species sensitivity to hypoxia, i.e. croaker > spot = blue crab > hogchoker ≈ mantis shrimp. Water column destratification and reaeration occurred with spring tide or strong winds and all species except the burrowing mantis shrimp returned to the deeper strata, indicating a preference for the deeper habitats.

Diel vertical migration of copepods *Acartia tonsa* and *Oithona colcarva* is disrupted by hypoxia (Roman et al., 1993). In mid-Chesapeake Bay during the summer, these copepods typically occur near the bottom during the day and migrate to the surface waters at night. However, when DO concentrations fell below 1 mg/L in subpycnocline waters, the copepods were displaced to the pycnocline, where the highest numbers were

found both day and night. When mixing occurred during the summer, the bottom waters were reaerated, and the copepods once again were found at depth during the day. Vertical migration is believed adaptive in that it places the copepods in the chlorophyll maximum at night to maximize food intake, yet it provides day-time avoidance of the surface waters, protecting the copepods from visual feeding bay anchovy.

The consequences of hypoxia on recruitment were examined for two species at a mid-Chesapeake Bay site: the naked goby *G. bosc*, a benthic oyster reef fish, (Breitburg, 1992), and Eastern oyster *C. virginica* (Osman and Abbe, 1994). In the naked goby study, low DO episodes were short-lived, but extreme (<0.5 mg/L), the result of movement of deep, oxygen-depleted bottom water into the near shore reef habitat. Following each severe intrusion, the naked goby population density fell dramatically at the deeper stations, which experienced the lowest DO (0.4 mg/L). Small, newly recruited, juveniles were absent, presumably due to extremely high mortality. There is evidence, based on observed densities, that older juveniles and adults survived these events by temporarily moving to inshore portions of the reef where DO was not as low, then return during the weeks following the event. Embryonic development was also affected. Males abandoned egg-containing tubes placed at deeper sites, and the majority to all of the embryos were dead. In addition, the youngest embryos collected from the shallower, less hypoxia-stressed site developed abnormalities following laboratory incubation. The severe intrusions occurred during peak periods of recruitment, with the lowest DO occurring on portions of the reef where recruitment was expected to be highest. These adverse effects were not observed at sites experiencing low DO $\geq$0.7 mg/L.

In the study with the eastern oyster *C. virginica* (Osman and Abbe, 1994), mortality was observed in newly-set (2 to 4 days old) animals during periods of prolonged intrusions of low DO water (<1 mg/L 40% of the time in bottom water during the first two weeks of two experiments). Mortality was proportional with depth, which corresponded to severity of hypoxia. Growth rate of surviving spat decreased after 1, 2, and 4 weeks following deployment, with a greater effect also occurring at the deeper stations. Survival and growth of juvenile oysters were unaffected following simultaneous deployment at the same stations, indicating greater tolerance of the older animals. The authors concluded hypoxia to be a plausible causative factor, acting directly or indirectly, although other causative factors also are possible.

Responses of the macrobenthic community to DO < 2 mg/L are reported for the lower Chesapeake Bay and tributaries (Dauer and Ranasinghe,1992; Diaz et al., 1992; Llansó,1992; Pihl, et al.,1991, 1992). Two community effects are reduced species number and abundance, with these effects increasing spatially and temporally with increasing severity and duration of hypoxia. There also is a shift with hypoxia from dominance of longer-lived, deeper burrowing species of a mature community to short-lived, shallow burrowing opportunistic species. The response of benthic species, and their subsequent recoveries following hypoxia, depends on species tolerance, the timing of the hypoxic event relative to larval availability and settlement, and life history strategy. Some infaunal organisms migrate towards the sediment surface with hypoxia, beginning around 2 mg/L (Diaz et al.,1992). Animals that migrate to the surface are exposed to predation by hypoxia-tolerant fish and crustaceans (Pihl et al., 1992). Defaunation may only occur

below 1 mg/L. These studies support 2 mg/L as the hypoxic effect threshold for the macrobenthos, which is consistent with the global literature (Diaz and Rosenberg, 1995).

To summarize, demersal finfish community biomass has been observed to diminish at DO <3.7 mg/L, and species richness to diminish at <3.5. These effects become increasingly pronounced with further DO decline. Below 2.0 mg/L, migration of the infaunal species to the sediment surface and movement of epifaunal species to better aerated water were observed. All effects reported at <1 mg/L DO concern hypoxia-tolerant species and life stages (i.e. disruption of diel vertical migration in copepods, reduced growth and survival of newly settled oysters, and lethality in larval goby) as demonstrated in parallel laboratory studies (Breitburg, 1992, Roman et al., 1993) or by other workers (Baker and Mann, 1992 and 1994a).

Data not used

Data from a variety of published literature were not used. The literature on effects of anoxia was not used, as it provides negligible information on threshold requirements of aerobic animals. Information on anoxic effects may be found in a recent symposium (Tyson and Pearson, 1991) and a review (Diaz and Rosenberg, 1995) of this subject. Results of hypoxia effects studies were not cited for species which do not commonly occur in coastal and estuarine waters between southern Cape Cod, MA and Cape Hatteras, NC during the spring to autumn period which brackets the occurrence of hypoxia. Reports for occasional visitor species that occur in these waters during a favorably warm or cold summer were excluded.

Data were not cited if the test temperature was outside the temperature range of Virginian Province waters during the hypoxic season, e.g. American lobsters tested at 5 ^{o}C (McLeese, 1956). Data were not used if they are probably not reliable. Examples include indications that the test animals may have been stressed, e.g. American lobster tested at 25 ^{o}C which were not fed during a 8-10 week acclimation period (McLeese, 1956); excessive control mortality (> 10% for juveniles or adults and > 20% for early life stages); the DO exposure concentration was uncertain, whether due to questionable DO measurements or failure to directly measure test chamber DO conditions (e.g. Reish, 1966); or if test animals were removed and handled during the test to make other measurements, e.g. for an energetics study (Das and Stickle, 1993). Literature on physiological responses of animals to hypoxia was reviewed, but was not found useful to determine low DO effect thresholds. See Herreid (1980) for a discussion of difficulties in using oxygen consumption results to describe DO requirements of invertebrates. Rombough (1988b) has developed an approach to identify the DO requirements for fish embryos and larvae, but this approach has not been employed with species applicable to Virginian Province saltwaters.

Some data are not used for juvenile blue crabs, *C. sapidus* (Stickle, 1988; Stickle et al., 1989). Effect concentrations for this species from this laboratory are an order of magnitude higher than values from an earlier study using adult *C. sapidus* (Carpenter and Cargo, 1957). In addition, these effect concentrations for juvenile blue crabs are almost all higher than values for larvae of all tested species. Another study (DeFur et al., 1990) showed that adult *C. sapidus* make respiratory adjustments that allow them to tolerate

long-term (25 days at 22 °C) exposure to 2.6 to 2.8 mg DO/L. These data for juvenile blue crabs are considered outliers until further testing shows otherwise.

Just prior to final completion of this document, a paper appeared (Secor and Gunderson, 1998) describing the effects of hypoxia and temperature on juvenile Atlantic sturgeon, *Acipenser oxyrinchus.* There was 22% mortality at 19 °C and an average within tank DO concentration of 2.7 mg/L (within tank data provided by author). This sensitivity is not that different from that of stripped bass. However, a combination of low DO (ca. 3.5 mg/L) and high temperature (26 °C) resulted in 100% mortality of *A. oxyrinchus* within approximately 24 hr. Because the greatest sensitivity was associated with the high temperature the data were not included in this document. In addition, the salinity during the experiments only ranged between one and three ppt, therefore it is likely that this data is more appropriately associated with freshwater criteria which are much higher than those for saltwater (see Implementation section).

National Criteria

The national criteria for ambient dissolved oxygen for the protection of saltwater aquatic life from Cape Cod to Cape Hatteras are summarized in Table 6 and presented graphically on Figure 20 (for persistent exposure) and Figure 21 (for episodic and cyclic exposure). These criteria are briefly described below:

(1) Protection of Juvenile and Adult Survival from Persistent Exposure
This limit is derived following the *Guidelines* procedures and is analogous the criterion maximum concentration (CMC), except that a protective DO concentration limit is expressed as a minimum as opposed to a maximum, as would be the case for a toxicant. This limit represents the floor below which dissolved oxygen conditions (for periods of > 24 hours) must not occur. Shorter durations of acceptable exposure to conditions less than the CMC have been derived from laboratory studies, as described in *(4)* below. Please refer to Table 1 for a detailed explanation of the derivation of this limit.

(2) Protection of Growth Effects from Persistent Exposure
This limit is derived following the *Guidelines* procedures and is analogous to the criterion continuous concentration (CCC) for a toxicant. This limit represents the ceiling above which dissolved oxygen conditions should support both the survival and growth of most aquatic species from Cape Cod to Cape Hatteras. Please refer to Table 2 for a detailed explanation of the derivation of this limit. This limit may be replaced with a limit derived in *(3)* as described below, when exposure data are adequate to derive an allowable number of days of persistent exposure.

(3) Protection of Larval Recruitment Effects from Persistent Exposure
This limit is derived from a generic larval recruitment model using data for the Say mud crab, a sensitive species native to the waters from Cape Cod to Cape Hatteras. It provides a degree of protection equivalent to the CCC described above in *(2)*. The limit represents allowable dissolved oxygen conditions below the CCC, provided the exposure duration does not exceed a corresponding allowable number of days that assure adequate recruitment during the recruitment season. The cumulative effects of all exposure interval dura-

tions at a given DO below the CCC can be accounted for by totaling the fractions of the actual (or projected) exposure duration (in days) divided by the allowable exposure duration for each interval of a specific DO concentration. Please refer to Table 3 and Figure 6 of this document for a detailed explanation of the derivation of this limit.

(4) Protection of Juvenile and Adult Survival from Episodic or Cyclic Exposure
This time dependent limit was derived to represent the responses of the most sensitive juveniles tested in the laboratory. It provides an equivalent degree of protection as the CMC, but for shorter exposure durations than a day. It is assumed that adults are no more sensitive than juveniles. This limit represents the minimum dissolved oxygen conditions that must be maintained on an hourly basis (e.g., one-hour minimum, two-hour minimum, etc.). The limit applies to conditions occurring on a single given day; even if this limit is met, recurring exposure patterns still must be checked for agreement with the larval recruitment limit described in *(6)* below. Please refer to Figure 10 of this document for a detailed explanation of the derivation of this limit.

(5) Protection of Growth Effects from Episodic or Cyclic Exposure
This limit is derived from the dose-response relationship for DO vs. growth reduction for the Say mud crab, and comparisons of the effects of cyclic exposure versus constant exposure on growth for a variety of species. It provides an equivalent degree of protection as the CCC, but for shorter exposure durations than a day. The limit represents the DO conditions that maintains a daily percent growth reduction in Say mud crab not greater than the level provided at the CCC for whole day exposures (23%). The cumulative effects of all exposure interval durations at a given DO below the CCC are accounted for by summing the percent reductions for time intervals at representative D.O. concentrations. An adjustment factor of 1.56 was derived to estimate time-variable effects from intermittent exposure tests that indicated residual, or delayed recovery effects from various growth-inhibiting conditions. The limit applies to conditions that may occur as a recurring pattern throughout the year without adverse growth effects at the CCC level of protection. However, a recurring pattern of exposure may be limited for a certain number of days based on the larval recruitment limit *(6)*. Recurring patterns of DO conditions that do not meet the growth limit may be allowed for a limited number of days in a recruitment season, provided the larval recruitment limit is met according to *(6)*. Please refer to Table 4 and Figure 12 of this document for a detailed explanation of the derivation of this growth limit. The larval recruitment limit can be substituted in whole for the growth limit.

(6) Protection of Larval Recruitment Effects from Episodic or Cyclic Exposure
This limit is derived from the modeled relationships between daily cohort mortality for the Say mud crab and the allowable number of days at a given maximum daily cohort mortality that protects against greater than 5% cumulative impairment of recruitment over a season. It provides an equivalent degree of protection as the limits described in *(3)* above, but for recurring patterns of low DO as opposed to continuous low DO conditions. Figure 16 of this document illustrates how to determine the maximum daily cohort mortality from duration intervals of DO minima. Figure 17 of this document illustrates how to determine the allowable number of days of cyclic exposure for a given maximum daily

cohort mortality. This limit provides additional information that should be used in conjunction with the limits described in *(4)* and *(5)* above. The limit determines the number of days that recurring episodic or cyclic conditions may occur, including whether the pattern may occur for an unlimited number of days. For example, a cyclic pattern that includes a DO minimum of 3.6 mg/L for 8 hours results in a daily cohort mortality of 10% (see Figure 16). Assuming this represents the maximum daily cohort mortality for the cyclic pattern, the allowable number of days for the cyclic exposure is 26 (see Figure 17). Please refer to pages 31-34 of this document for a detailed explanation of the derivation of this limit.

In summary, limits (1) and (4) establish one day and hourly minimum conditions that should be maintained for persistent and cyclic exposures, respectively; limits (3) and (6) establish conditions that may occur for a limited number of days for persistent and cyclic exposures, respectively; and limits (2) and (5) establish long term conditions that should be maintained for the remaining number of days for persistent and cyclic exposures, respectively.

Implementation

Dissolved oxygen criteria should be implemented differently from those of toxicants, but not for reasons associated with biological effects or exposure. Uncertainties associated with aquatic effects of DO, such as behavior, synergistic relationships with temperature, salinity, or toxics, apply to toxics as well. Dissolved oxygen also does not differ from toxics for reasons associated with exposure. Dissolved oxygen can vary greatly in the environment, but so can toxics. Effluents and their receiving waters can vary daily, even hourly, in their toxicity to aquatic life. Toxicity of saltwater receiving waters also can vary with the tide and the depth of water. It may be mistakenly perceived that DO varies more in concentration simply because it can be measured easily and nearly continuously.

From the standpoint of environmental management, DO differs from toxic compounds primary because it is not regulated directly. Hypoxia is a symptom of a problem; not a direct problem. Dissolved oxygen is regulated primarily by controlling discharges of nutrients (in the marine environment, most commonly nitrogen). Dissolved oxygen also differs from most toxic compounds because hypoxia can have a large natural component. Therefore, criteria for hypoxia should not automatically be applied in the same way as limits for toxicants are.

This document provides the information necessary for environmental planners and regulators in the Virginian Province to address the question of whether DO at a given site is sufficient to protect coastal or estuarine aquatic life. The document does not address how compensatory mechanisms such as avoidance can influence the response of local populations to seemingly adverse DO conditions. The document also does not address the issue of spatial extent of a DO problem. In other words, even if the DO at a site is low enough to significantly affect aquatic life, the environmental manager will have to judge whether the hypoxia is widespread enough for concern.

Table 6. Summary of Saltwater Dissolved Oxygen Criteria.

	Persistent Exposure (24 hour or greater continuous low DO conditions)	Episodic and Cyclic Exposure (less that 24 hour duration of low DO conditions)
Juvenile and Adult Survival (minimum allowable conditions)	*(1) a limit for continuous exposure* DO = 2.3 (mg/L) (criterion minimum concentration, CMC)	*(4) a limit based on the hourly duration of exposure*. $DO = 0.37*\ln(t) + 1.095$ *where*: DO = allowable concentration (mg/L) t = exposure duration (hours)
Growth Effects (maximum conditions required)	*(2) a limit for continuous exposure* DO = 4.8 (mg/L) (criterion continuous concentration, CCC)	*(5) a limit based on the intensity and hourly duration of exposure.* Cumulative cyclic adjusted percent daily reduction in growth must not exceed 23%. $\sum_{1}^{n} \frac{t_i * 1.56 * G_{red_i}}{24} < 23\%$ *and* $G_{red_i} = -19.4*DO_i + 116.2$ *where:* G_{red_i} = growth reduction (%) DO_i = allowable concentration (mg/L) t_i = exposure interval duration (hours) i = exposure interval
Larval Recruitment Effects[1] (specific allowable conditions)	*(3) a limit based on the number of days a continuous exposure can occur* Cumulative fraction of allowable days above a given daily mean DO must not exceed 1.0 $\sum_{1}^{n} \frac{t_i^{(actual)}}{t_i^{(allowed)}} < 1.0$ *and* $DO_i = \frac{9.57}{\left(2.15 + 2.3e^{-0.16t_i}\right)}$ *where:* DO_i = allowable concentration (mg/L) t_i = exposure interval duration (days) i = exposure interval	*(6) a limit based on the number of days an intensity and hourly duration pattern of exposure can occur.* Maximum daily cohort mortality for any hourly duration interval of a DO minimum must not exceed a corresponding allowable days of occurrence. *where:* Allowable number of days is a function of maximum daily cohort mortality (%). Maximum daily cohort mortality (%) is a function of DO minimum for any exposure interval (mg/L) and the duration of the interval (hours).

[1] model integrating growth and survival effects to maintain a minimally impaired Say mud crab larval population

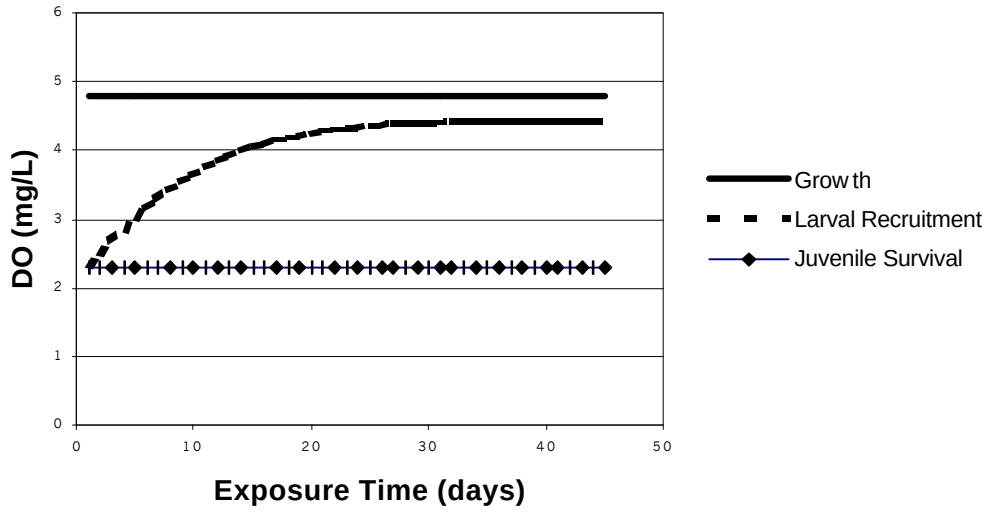


Figure 20. Summary of Criteria for Persistent Exposure. The larval recruitment line represents the minimum DO concentration that may persist for a given exposure interval duration (number of days). The cumulative effect of multiple intervals during a season must be accounted for as described in *(3)* above and in the equation provided on Table 6.

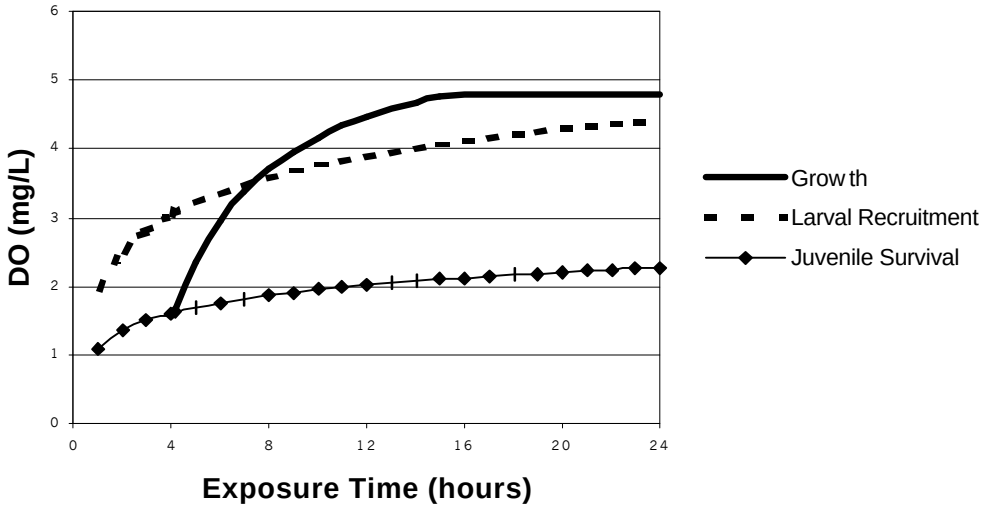


Figure 21. Summary of Criteria for Episodic and Cyclic Exposure. The growth line represents the minimum DO concentration that may persist for a given exposure interval duration (i.e., the exposure duration/DO concentration that results in a 23% daily growth reduction). The cumulative effect of multiple intervals during the course of a day must be accounted for as described in *(5)* above and in the equation provided on Table 6. The larval recruitment line represents the hourly exposure duration/DO concentration intervals that may recur for an unlimited number of days (corresponds to the 8% daily cohort mortality as shown on Figure 17).

Finally, as with all criteria, this document does not address changes in sensitivity to low DO that accompany other stresses such as high temperature, extremes of salinity, or toxicants. Chief among these concerns would be high temperature because high temperature and low DO often appear together. Low DO will be more lethal at water temperatures approaching the upper thermal limit for species. This effect has been seen for freshwater species (U.S. EPA, 1986; Secor and Gunderson, 1998), and saltwater species (e.g., *C. irroratus* and *E. affinis*). The limits provided here should be sufficient under most conditions where aquatic organisms are not otherwise unduly stressed.

Many programs that monitor coastal DO with electronic equipment cannot measure DO to better than 0.5 mg/L due to limitations of instrument accuracy and resolution (e.g., Strobel, et al., 1995; Strobel and Heltshe, 1999) or sampling design (Summers, et al., 1997). Attempts to refine the limits presented here or to apply these limits in assessing field DO conditions should take this into account. Criteria for DO can be used in a risk assessment framework. The approach outlined in this document can be easily used to compare among areas the DO conditions that are adequate to support aquatic life. Environmental managers can determine which sites need the most attention, and evaluate the spatial and temporal extent of hypoxic problems from one year to the next for sites of major concern.

Environmental managers who wish to use the protective approach presented here will have to decide several questions about how the limits will be used, four of which are described below.

1. *Accuracy of monitoring data*—The most important decision is to determine how accurate the monitoring data are—the better that hypoxia is characterized, the more reliably it can be decided whether it meets the criteria. Data from existing monitoring programs may not always be accurate enough to take full advantage of the approach provided here. For example, a recent assessment of conventional sampling procedures along the Atlantic and Gulf coasts has suggested that hypoxia in estuarine waters is substantially more widespread that previously believed (Summers, et al., 1997). Deciding what data can adequately characterize hypoxia is a matter of risk management. Cyclic conditions may require measurements every 30 min for several days, whereas persistent hypoxia may need only several measurement a week. Decisions also have to be made about the number and locations of sampling sites to properly represent a given area.
2. *Biological effects*—Potential biological effects are most difficult to predict when DO lies between the limits for juvenile and adult survival and larval growth. Concentrations below the juvenile and adult limit do not protect; concentrations above the limit for growth probably protect most aquatic life and its uses[15]. Deciding whether concentrations between the limits are acceptable will depend on the duration of hypoxia and on the acceptable impairment of larval recruitment. The acceptable impairment can be a risk-management de-

[15] The larval growth protection limit is based on statistically significant differences that result in chronic values similar to EC25s for growth of many organisms. EC25 values are listed as a part of Appendix C for four species of crustaceans and two species of fish. The geometric mean of these values (by species) correlates with the geometric mean of the chronic values.

cision. The 5% impairment level was selected to be consistent with the protection provided to juvenile and adult life stages. In addition, a model that integrates gradual change in daily DO conditions may more accurately predict recruitment effects than the current simplified model and its application.

3. *Spatial extent*—After environmental managers have found a hypoxic area, they must decide whether it is small enough relative to nearby unaffected areas to allow the coastal region as a whole to meet the criteria.
4. *Freshwater versus saltwater*—It is not trivial to decide whether the DO in certain parts of estuaries should be judged by freshwater criteria or saltwater criteria, particularly where the tides vary the salinity between near fresh and a few parts per thousand. This decision is important because the criteria for freshwater can be up to twice as great as the saltwater limits developed here, depending on water temperature and the life stage being protected (U.S. EPA, 1986). A reasonable way to start is by considering an estuary's biological communities. If they are more like freshwater organisms, freshwater criteria should be applied. If they are more like saltwater, then saltwater criteria apply.
5. *Threatened or endangered species*—In cases where a threatened or endangered species occurs at a site, and sufficient data exists to suggest that it is more sensitive at concentrations below the criteria, it is appropriate to consider development of a site-specific criterion.

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